ANNUAL REPORT
OF
PROGRAM ACTIVITIES
NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT
FISCAL YEAR 1979

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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ANNUAL REPORT

OF

PROGRAM ACTIVITIES

NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT

Fiscal Year 1979

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National Institute of Child Health and Human Development

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NICHD ANNUAL REPORT

October 1, 1978, through September 30, 1979

OFFICE OF THE DIRECTOR

The National Institute of Child Health and Human Development supports and conducts a program of research unique to the western world in combining attention to maternal and child health and a full range of population studies. It focuses on the special health problems of women, children and families and the means to prevent childhood conditions that result in disease, disability, and the major killers and cripplers of adult life.

To encompass the diversity required to address these problems, the NICHD has developed a research strategy based on a multidisciplinary mix of studies in reproductive and developmental biology and the behavioral and social sciences. Commitment to this strategy directs the Institute's efforts in biomedical and behavioral research to these goals:

> to insure the birth of healthy babies through investigations on prenatal and infant nutrition, normal and abnormal fetal development, and problems of pregnancy and birth;
>
> to insure the birth of wanted babies through studies on human fertility and infertility, contraceptive development and safety, and the reduction of unwanted pregnancies, particularly among adolescents; and
>
> to provide all children an opportunity for productive and healthful adulthood through research to prevent childhood problems such as smoking, obesity, environmentally induced mental retardation, and learning disorders that contribute to adult disease and disability.

The scientific knowledge that underlies these goals fall into two broad areas: reproductive studies and research in the developmental sciences, including studies in the behavioral and social sciences. Reproductive studies range from research on immunological methods of fertility regulation to studies of population and family dynamics. Research in the developmental sciences covers a broad range, from studies in human embryology to investigation of how children learn and develop.

This report, which recounts intramural and contract supported research projects of the Institute, is testimony to the scope and variety of the NICHD program. Last year's optimistic report was that Congressional appropriation of $37 million for construction of a Child Health Intramural Research Facility (Building 33) would provide a first-class research environment for developing a cohesive and expanded intramural research effort. Unfortunately, in an economy move, the appropriation was rescinded. The Congress also directed the Institute to perform a study, and report by January 1979, on the need for and feasibility of establishing a clinical research center for its programs. The directive required identification of the functions and programs that would be carried out in such a center, the number of square feet required,

an appropriate geographical location, and the most economical way to establish such a center. Institute staff and a task force of consultants have submitted the report.

The adjustment to static funding levels and, in some cases, reduction of funds, inhibit development of new initiatives, and the assessment and evaluation of programs assumes new importance. Development of long-range plans will be required, furthermore, to cope with decreased growth. In the meantime, we are pleased with the many positive accomplishments described in the following record.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Center for Population Research
Office of the Director

Introduction: As the Institute's Center for Population Research (CPR) passed the tenth year of operation since being established in August 1968, it continued to fulfill its responsibility for the primary Federal effort in population research. Significant progress has resulted again this year from the Center's support of research in the population sciences through grants and contracts for:

-Fundamental biomedical research in the reproductive sciences relevant to problems of human fertility and infertility.

-The development of new and improved methods for fertility regulation.

-The evaluation of the safety and effectiveness of contraceptive methods currently in use.

-Social and behavioral sciences research on the reproductive motivation of individuals, and the causes and consequences of population change.

Research in the reproductive sciences on fertility and infertility is concerned with the reproductive processes involved in the fertility of men, women, and animals with reproductive processes similar to those in human beings. This program in fundamental biomedical research includes studies on the development of the egg and sperm, ovulation and its regulation, fertilization, the mechanism of hormone action, neuroendocrine control of reproductive processes, reproductive diseases, and the relationship between nutrition and reproduction. CPR, NICHD is the largest single source of support for such research. Studies of reproductive biology in the human and relevant animals are supported in order to increase our knowledge of reproductive processes, the regulation of fertility, the alleviation of infertility, and the amelioration or cure of diseases and disorders of the human reproductive system. The primary goal of these efforts is to enable men and women to have healthy babies in the number and at the times such babies are wanted.

The program to improve methods of fertility regulation involves efforts to develop new drugs, devices, and other means of contraception, for both men and women, which are safe, effective, inexpensive, reversible, and acceptable to various population groups. The objective is to develop an array of methods that can be used successfully by different couples under various circumstances. Research activities to develop a variety of safe contraceptive methods include synthesis and biological evaluation of promising new compounds for the female or male; development of technology for improved drug administration; development of improved vaginal and uterine contraceptives based on chemical or physical methods; clinical trials of sex steroids for suppression of sperm production and the consequent development of chemical contraceptives for males; clinical

and toxicological evaluation of long-acting progestin as a female contraceptive; laboratory studies and clinical trials to develop and ascertain the efficacy of antifertility methods based on periodic abstinence; and studies required for clarification of mechanisms of action of specific drugs. At all the stages of research on contraceptive development, the overall aspect of product and method safety is emphasized.

The program in contraceptive evaluation is concerned with the evaluation of the safety of various contraceptive methods which are currently in use, particularly with respect to long-term safety. Efforts are directed toward attaining improved safety of fertility regulation methods through identification of health risks associated with methods currently in use, delineation of particular circumstances which increase the risks of specific methods, and clarification of the mechanisms by which specific adverse effects of methods currently in use arise. The scope of the program includes the evaluation of health hazards associated with oral contraceptives and of intrauterine devices, and also the immediate and long-term risks associated with methods of sterilization for men and for women. In addition, studies are supported to ascertain the possible adverse consequences of pregnancy termination for the health of women, particularly regarding subsequent pregnancy performance. Among possible hazards or suspected health risks of steroid contraceptives being studied are cancer and other forms of tumors, heart disease, hypertension and thromboembolic disease, metabolic and nutritional disorders, birth defects, and also the understanding of the underlying mechanisms by which known complications arise.

Research in the social and behavioral sciences involves the factors governing variations in the growth, distribution, and characteristics of people and the impact of population changes on the health and welfare of individuals, families, and society as a whole. Studies are supported on the demographic, social, behavioral, and economic factors which influence variations in family size among individuals and groups and the causes and consequences of varying levels of fertility for the population as a whole or segments of it. Emphasis is given to research on motivation for fertility regulation; variations in the perceived value and costs of children; the determinants and consequences of problems associated with adolescent pregnancy and childbearing; effects of changes in the structure, functions, and size of the family; factors affecting the adoption and effective use of contraception on the part of both men and women; and the related effects of changing roles in our society, particularly of women. Studies concerning the causes of fertility trends in the United States are also emphasized because they identify changes occurring by age, region, and subgroups of the population. The knowledge resulting from these research efforts is necessary for the formulation of desirable public policies and goals, as well as for the means to achieve them, and for assessing their impact.

Recent findings and achievements in population research supported by the Institute include the following:

1. Significant advances were made in studies using the estrogen-ovalbumin system of the chicken as a model for hormonal regulation of genetic expression. The complete molecular organization of the DNA sequences composing the ovalbumin gene was reported.

2. Progress was made in mapping the fine-structure of gene variations associated with reproductive processes and their possible disorders. This work has provided a basis for explaining molecular variations within genes which determine alternative hereditary characteristics in the ovalbumin gene of the chicken.

3. Research accomplishments characterizing the biochemical milieu of the developing sperm have added new knowledge to our understanding of male reproductive processes.

4. Several studies have shown that intermittent administration of low doses of LRF agonists results in clinical improvement in patients with certain reproductive disorders by improving gonadal function. LRF (luteinizing hormone releasing factor) stimulates the secretion of pituitary gonadotropins necessary for normal reproductive functions.

5. Preliminary data indicate that LRF exerts a direct effect on ovarian and testicular function, in addition to its well-known gonadotropin releasing action in the pituitary. These data suggest new approaches for possible application to fertility regulation.

6. The synthesis and biological evaluation of new analogs (chemical modifications) of the hypothalamic gonadotropin releasing hormone has resulted in drugs that are much more potent and longer acting than the natural hormone. Preliminary human data has established their utility as ovulation suppressants and luteolytic agents for potential use in fertility regulation.

7. Considerable progress has been made on the development of contraceptive drug delivery systems including the implantable caprolactone polymer system, the microcapsular injectable system, and the bioerodable system using the polymer, Chronomer.

8. Clinical trials of two methods of periodic abstinence (natural family planning) have been completed. The sympto-thermal method was clearly superior to the ovulation method in terms of use-effectiveness.

9. The program to distribute reagents developed with CPR support to scientists around the world for research on reproductive processes has been very active. The primate reagent efforts, such as the collection of monkey pituitary glands for extraction of hormones, are very significant since the importation of primates is being curtailed and the national breeding program cannot meet the current demand.

10. A large study of the relative risk of a heart attack for women using oral contraceptives has confirmed previous observations. An increased relative risk was observed principally among women on the pill who had other important

risk factors for cardiovascular disease, primarily cigarette smoking.

11. An increase in the extent and severity of atherosclerosis was found in the major arteries of rhesus monkeys that had been vasectomized 9 to 14 years previously, in comparison to age-matched controls.

12. Research has revealed a small but significant increased risk in the incidence of second trimester spontaneous abortions in women with one or more prior induced abortions.

13. Improved control of fertility has reduced the incidence of unintended births in the United States, and sterilization is becoming the major method of limiting family size.

14. Preliminary findings show that spouses who disagreed regarding number and spacing of children, when compared with those agreeing, desired a larger family size; discussed family size and sexuality less frequently; and communicate feelings less frequently.

15. While birth rates of older adolescents have declined in recent years, birth rates of middle adolescents have remained fairly high and the rates have increased among younger adolescents.

16. Several studies have documented the effect of an early first birth on subsequent fertility showing that the woman who begins her childbearing as an adolescent is more likely to have a larger number of children, more unwanted births, more out-of-wedlock births, and a faster pace of subsequent childbearing.

17. Research examining the child's later development has shown that the mother's age at her child's birth and social factors are related to the child's subsequent physical health, cognitive and social development. The social, emotional, and intellectual development past infancy of the children of adolescents continues to be related to the mother's age at birth.

18. A comparison of national data from surveys done in 1971 and 1976 shows that the use of effective methods of contraception has increased among unmarried teenage girls, but there are still many who do not use contraception.

19. A study has shown that on the basis of fertility rate changes between 1960 and 1974, one-child families in the U.S. are increasing.

20. Presently, legal immigration accounts for one-fourth of the yearly population increase observed in the U.S. and illegal immigration is estimated to contribute even more.

Coordination and Communication

The activities of the Center to enhance the coordination of Federal population research programs and to foster the communication of biomedical and behavioral research information in the population sciences, are presented

in this portion of the report since they are coordinated in the Office of the Director.

Interagency Committee on Population Research (ICPR), which is chaired by the Director of CPR, coordinates the population research activities supported by Federal agencies and facilitates the exchange of information and ideas among Federal programs involved with population research. The ICPR, established by the Secretary of HEW on October 5, 1970, has been extended by the Secretary through June 30, 1980. It is comprised of representatives from the various Federal agencies concerned with research related to human population problems. The major products of the ICPR are the following two publications produced annually with the assistance of NICHD's Office of Planning and Evaluation.

Inventory and Analysis of Federal Population Research for fiscal year 1978 was issued by the ICPR. This report contains (1) a description of the background and role of the ICPR; (2) a statistical analysis with fiscal tables by research areas and supporting agencies; (3) an evaluative analysis with recommendations for needed population research; (4) a detailed inventory of all Federally supported population research projects classified according to the major research category; (5) a listing of projects started in FY 1978; and (6) a membership list of the ICPR.

Inventory of Private Agency Population Research for calendar 1977 was published by the ICPR. This report provides information on the population research projects sponsored by the major U.S. private organizations in this field. These three principal organizations involved with research in the population sciences, the Ford and Rockefeller Foundations and the Population Council, have provided the data on their research projects in the same way as the Federal agencies and the information has been published in the identical format as the Federal Inventory.

Population Sciences: Index of Biomedical Research continues to provide a useful resource to those who wish to be currently informed about the biomedical literature in the population research field. This monthly bibliographic citation journal began its sixth year of publication in October 1978. It is produced with the cooperation of the National Library of Medicine based on the information contained in their Medical Literature Analysis and Retrieval System (MEDLARS).

Population Research Monographs published by the Center through the Government Printing Office are another means for furthering the dissemination of information. These books provide a comprehensive review of the state-of-the-art in specialized areas of population research in the biomedical or social sciences and indicate future research directions to obtain needed knowledge. The Center's conferences and workshops in the population sciences also continue to result in publications which constitute a valuable addition to the population research literature.

Ovarian Follicular Development and Function, edited by Dr. A. Rees Midgley of the University of Michigan and Dr. William A. Sadler, Chief of CPR's Population and Reproduction Grants Branch, reports the proceedings of the Workshop sponsored by the Center and held at the University of California at Santa Cruz. It is concerned with the processes of hormonally and nonhormonally controlled progressive functional and morphologic differentiation of thecal cells, granulosa cells, and oocytes; exogenous and endogenous hormonal regulation of receptors for protein and steroid hormones; and the eventual death through atresia or rescue by ovulation and luteinization. The book has been published by Raven Press.

Adolescent Sexuality in a Changing American Society, written by Dr. Catherine Chilman of the University of Wisconsin, presents an analytic overview and summary of research related to the social and psychological aspects of adolescent sexuality. It contains a critique of the available research, speculations about its meanings, and suggestions for needed future studies. Among the topics included in this book are: a condensed summary of the major aspects of biological, social, and psychological knowledge and theory concerning adolescent sexuality; a discussion of the central features of the society with which contemporary adolescents interact; sexual behaviors and attitudes of the developing adolescent; contraceptive attitudes and behaviors; illegitimacy among adolescents; adolescent abortion, teenage marriages, and childbearing within marriage; and implications for future research, policies, and programs. It was prepared under contract with CPR's Behavioral Sciences Branch and has been published by the Government Printing Office.

The Economic Consequences of Slowing Population Growth, edited by Drs. Thomas Espenshade and William Serow, resulted from a CPR-sponsored conference of the same title. The book presents a demographic and economic analysis of the implications of the current trends of slowing population growth among the developed countries. It includes U.S.-European comparisons, analyses of the implications for economic growth, employment, income distribution and public sector activity, and a policy summary as well as a comprehensive bibliography. It was published by Academic Press.

Vasectomy: Immunologic and Pathophysiologic Effects in Animals and Man, edited by Irwin H, Lepow and Ruth Crozier, reports the proceedings of a workshop organized by the Contraceptive Evaluation Branch and held at Airlie, Virginia, in 1978. The book presents data from experimental and clinical studies in a wide range of disciplines and includes reports on local and systemic effects, endocrine function, immune reactions, pathologic findings, and clinical and epidemiological studies of possible medical sequelae. It was published by Academic Press.

Ten Year Progress Report of the Center for Population Research, describes many of the achievements of research supported by CPR, NICHD since its establishment in August 1968. It contains reports on Contraceptive Development, Contraceptive Evaluation, Behavioral Sciences, and Coordination and Communication covering the past ten years and for Fundamental Research in the Population Sciences and Institutional Support for the last five years since Dr. Sadler was appointed Branch Chief for this area.

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October 1, 1978 through September 30, 1979
Center for Population Research
Contraceptive Development Branch

In fiscal year 1979, attempts were made to consolidate efforts toward development of available leads in several program areas. Sufficient data have been accumulated to permit exploration of certain research endeavors in the human. As a consequence of increased activity in the product development area, and consonant with established policy, there has been a concomitant reduction in support of directed basic research.

A. Product Development

Drug Development Program

The synthesis and biological evaluation of new analogs of the hypothalamic gonadotropin releasing hormone has been the subject of intensive research efforts. In the agonist area, numerous superactive analogs have been prepared. These drugs are much more potent and longer acting than the native hormone. The pragmatic utilization of these drugs for fertility regulation in both the female and the male has been strengthened by a better understanding of the paradoxical effects exhibited by these drugs. Repeated injections of agonists result in decreased pituitary function and possibly direct effects on the ovary and testis. Preliminary human data established the utility of agonists as ovulation suppressants and luteolytic agents. Based on data from laboratory animals and human clinical data, plans were implemented to more fully explore the potential of the agonists for fertility regulation. The initial endeavors will focus essentially on the safety and mechanism of action of agonists, and when these have been established clinical trials for efficacy will be implemented.

In the antagonist area, major breakthroughs in potency of new analogs have been achieved. With the antagonist [D-Ac-Phe^{1}, D-p-Cl-Phe^{2}, D-Trp^{3,6}] LHRH a single 30 μg dose given during proestrus inhibits ovulation in the rat. This breakthrough has been achieved through very close monitoring of the various contracts and the rapid feedback and sharing of biological data obtained from our testing facility. It is important to further enhance the biological potency of the antagonists in order to reduce the total amount of drug necessary to accomplish the desired biological effect and to improve chances for eventual oral activity.

In the prostaglandin area, efforts have been continued to produce a safe luteolytic agent. Fluorinated derivatives of the prostaglandin analogs appear to significantly decrease smooth muscle stimulation when compared to the nonfluorinated derivatives and therefore lend encouragement to the notion that luteolytic and smooth muscle stimulating activities can be separated. Continuation of efforts from prior years has helped in focusing on improved animal models for assessment of biological activities. The issue of animal models is quite critical to proper assessment of biological activities. Activity has continued in the synthesis of 7,11-substituted androgen derivatives which show enhanced biologic activity. In the estrogen area, interesting biological activity was observed in 11-substituted estrone and estradiol derivatives. These compounds

do not possess the usual 17α-ethynyl function associated with oral activity and yet show profound activity upon oral administration. Since a number of undesirable side effects have been associated with the estrogenic component of oral contraceptives, an effort is being instituted to assess the safety of the new estrogens.

Synthetic Chemical Facility

The synthetic facility continues to fulfill its important role of providing a variety of required services. The facility has participated in the synthesis of (1) novel compounds, (2) known, but commercially unavailable, compounds, (3) chemical intermediates required for other synthesis contracts, (4) compounds labeled with radioactive and stable isotopes, and (5) resolution of racemates. Since the facility meets the Good Manufacturing Practices standards, it is in a position to provide the Branch with materials that are needed for animal and clinical studies.

Biological Drug Testing Facility

The drug testing program is committed to rapid evaluation of compounds and drug delivery systems. During this fiscal year it has been operating at its full capacity. Additional capabilities have been acquired through the development of an in-house radioimmunoassay (RIA) facility. When fully operational, the RIA facility will permit evaluation of drug and hormone levels in test animals.

The drug testing program was kept busy assessing the biological activity of the numerous LH/FSH-RH analogs. Different bioassays are providing data on compounds originating from several synthesis contracts. The program actively augments biological studies conducted by other contractors.

Screening programs for long-acting androgens and progestins have been expanded. Conduct of these assays has been optimized to permit better interpretation of the results. Data storage, retrieval and analysis have been computerized. Consequently, rapid comparative evaluation of the various drugs can be performed.

Drug Delivery Systems and Oral Formulations

Solid progress has been accomplished in all phases of drug delivery systems. Data on the implantable caprolactone polymer system are being collated for submission to the FDA. Animal safety data strongly suggest that the system is safe. Clinical protocols for Phase I studies have been prepared, and the studies will commence after an IND is obtained from the FDA.

The microcapsular injectable system developed by one of our contractors is being compared to another system developed by outside sources. The comparative study should permit selection of a system that meets certain criteria. Assuming that well-controlled release rates can be achieved by both preparations, selection of one of the systems for intensive investigation will depend on which one of the systems offers the more pragmatic manufacturing technology.

Development of the bioerodable system employing Chronomer® is being optimized. Improved manufacturing techniques to produce large batches of the polymer have

been accomplished. A number of minor problems that have surfaced during the year have been resolved, and the necessary animal safety studies will be implemented. The Chronomer® program is benefiting substantially from the WHO interaction. Certain phases of research which were sponsored by WHO are more advanced than the ones supported by NIH, and consequently improved program planning can be implemented in the joint program.

The overall nature of drug delivery systems was the subject of a two-day workshop sponsored jointly by NIH and WHO. One of the recurrent problems experienced with long-acting drugs and systems is their potential for altering menstrual cycle phenomena. The major issues in patient acceptability have been breakthrough bleeding and amenorrhea. Although neither one of these conditions is desirable, the reaction of different population groups varies. Experience with the tested systems and drugs strongly suggests that among American women amenorrhea is more acceptable than irregular menses. This type of knowledge dictates that higher blood drug levels will be required to achieve amenorrhea. One additional factor which was the subject of extensive discussion involves the retrievability of the system under conditions where it is medically indicated. The implantable systems are more difficult to administer, but can be retrieved. The injectables offer ease of administration but are difficult, if not impossible, to retrieve. Keeping these considerations in mind, it is projected that the implantable systems will be designed to deliver drugs over more extended periods of time than the injectables.

The first phase of experiments designed to modify the absorption pattern of currently available oral contraceptives has been completed. Two different formulations have been prepared which slowly eliminate the sharp initial peak without reducing later blood levels of the active components. The overall lower blood levels may result in improved safety characteristics for the available drugs. Following consultation with the FDA, a decision will be made whether human experimentation may be initiated or whether additional animal safety studies need to be undertaken.

Device Development

Clinical studies of the estriol releasing IUD have been initiated and should be completed at the end of FY 1980. It is hoped that these studies will clarify the issue of whether estriol in a continuous release mode can or will not induce endometrial hyperplasia.

Development of improved barrier methods of contraception has not progressed satisfactorily. We have not been successful in eliciting proposals that merited funding. From a historical perspective this type of situation has arisen before in other research areas and has been resolved via additional program solicitations. The importance of barrier methods in fertility regulation must be underscored at the time when the safety of the more effective methods is questioned.

Clinical Studies (Female)

Clinical studies on norethindrone enanthate have been temporarily suspended pending the FDA decision on the drug safety. Resolution of this impasse is anticipated at the end of the current fiscal year. Data being generated by

WHO continue to show high efficacy with the once-every two-months injection schedule. We continue to be encouraged by the absence of serious problems in the large population of women to whom the drug has been, and is being, administered.

Clinical trials of two methods of period abstinence, referred to as natural family planning (NFP), have been completed. The Sympto-Thermal Method (ST) of NFP was clearly superior to the Ovulation Method (OM) in terms of use-effectiveness. This superiority was striking in all of the analyses presented: net rates, gross rates, and Pearl rates measured either from the beginning of training or from formal study entry showed at least a 2-1 advantage of ST over OM after 12 months of experience. Total dropout rates were high for both methods of NFP. Half of the women who started training dropped out, and half of those who completed training and entered the study dropped out within the first year. Voluntary termination of couples during training and in formal study was high. Lack of interest/dissatisfaction with the method was the major factor involved in voluntary termination during the training period. The dropouts and voluntary terminations are not necessarily reflective of the true status of NFP and may be partially accounted for by the demands imposed by the study. It can be concluded that improvements in the use-effectiveness of both methods should be anticipated with refinements of techniques for teacher and user training. Additionally, those subjects who select OM because of its simplicity may benefit from the use of basal body temperature and the calculated infertile phase while learning mucus observations.

Two additional studies in NFP methodology which are not directly involved with clinical trials need to be mentioned. Both of them involve analyses of menstrual cycle data obtained from subjects utilizing NFP for fertility regulation. In one study, basal body temperature (BBT) records have been subjected to computer analysis. Special programming is helping both the teacher and user in interpretation of the monthly cycle records. In the other study, an attempt is being made to relate various observations to the estimated time of ovulation as determined by hormone analysis. Both BBT and cervical mucus are related to the time of ovulation with mucus giving a somewhat more accurate retrospective signal than BBT.

A clinical trial of two vaginal barrier methods has been in progress for the past year. The study is attempting to assess the use-effectiveness of a foaming vaginal suppository (Encare-Oval®) and that of diaphragm in combination with a spermicide. The vaginal suppository has been widely advertised as safe, acceptable and effective; however, the supporting data have been marginal. Preliminary data strongly suggest that the acceptability of this specific suppository has been relatively poor because the product has produced vaginal and penile irritation.

Clinical Studies (Male)

In previously reported studies, it was observed that testosterone enanthate (TE) administered at frequent intervals, every seven to ten days, can drastically reduce sperm production. Less frequent injections are not as effective in spermatogenesis suppression. The action of TE appears to be closely related to the pharmacokinetics of this drug in man. Injection of nonlabeled TE (200 mg)

produces a rapid elevation in serum testosterone (T) with a peak at 24 hours. At one week after the injection there was still a 33 percent elevation in serum T, with a return to baseline at about nine days. Serum estradiol (E_2) paralleled the T levels, although the peak response lagged one day behind serum T. Serum LH and FSH exhibited a pattern opposite to that of serum T and E_2. These preliminary data help to explain the necessity for frequent TE injections and why spacing of injections at intervals greater than 10-14 days fails to maintain spermatogenic suppression.

Distribution Program

During FY 1979 the reagent distribution program was very active. Hundreds of investigators received products developed with CDB support. The distribution program is global in nature and represents the true spirit of international scientific collaboration. As part of this program, CDB and WHO are embarking on a joint venture to provide the scientific community with RIA reagents for nonhuman primate pituitary glycoprotein hormones. First priority is being assigned to the collection of monkey pituitary glands which will serve as the starting raw material for extraction of hormones. The primate reagent program is becoming more and more important in view of the fact that importation of primates is being curtailed and the national breeding program cannot meet the current demands. Reagents for which CDB is directly responsible include (1) LH-RH, (2) hCG, hCG subunits and antisera to them, (3) subunits of ovine LH, (4) RIA kits for nonhuman primate FSH, (5) PMSG, and (6) pregnancy diagnosis kits for nonhuman primates. The distribution program involves close collaboration among CDB, intramural NICHD program and NIAMDD.

B. Directed Fundamental Research

The number of projects falling in this category has become relatively small, and it is not projected that new projects will be initiated.

Ovulation Detection

The feasibility of detecting ovulation in women by the use of an indicator strip that changes color in response to an increase in the concentration of pregnanediol glucuronide (PG) in urine has been investigated. Observations that an increase in the concentration of PG in urine is parallel to the rise in serum progesterone after the preovulatory surge of LH support the concept of this research. The color change in the indicator strip will be based on uptake of fluoroscein-conjugated antipregnanediol antibodies in excess of that required to bind PG in urine samples. To this end, fluoroscein conjugation of the partially purified antiserum has been achieved without significant loss in binding activity.

RIA Development

One of the tools critically needed by the scientific community is a specific and sensitive RIA for monkey LH. Availability of this RIA in a kit form can be of great value in both basic and applied research. At this stage of development, the limiting factor appears to be availability of LH that can be used for radioiodination. A two-step approach is being taken to resolve this dif-

ficulty. First of all, numerous heterologous LH preparations are being screened against available antisera. As a backup to this approach, a purification program for monkey (m) LH is being instituted. In the latter approach there is good scientific evidence that if sufficient quantities of mLH are obtained, the problem will be resolved and a good RIA will become available. A wide-ranging program for monkey pituitary collection is being organized in order to secure current and future needs.

Follicular Dynamics

At birth the female mammal has the full complement of ova. The overall maturation process involving follicular growth is not fully understood. One of the approaches used to acquire the knowledge of follicular dynamics involves the counting of preantral follicles in ovarian sections obtained during defined stages of the rhesus menstrual cycles. During the follicular phase of the cycle preantral follicles 100 μm in diameter predominate. This is in contrast to the luteal phase when follicles with diameters of 50 μm are more prevalent. Another interesting observation is that preantral follicles tend to develop in clusters which are composed of follicles of various sizes. This observation seems unusual as the follicles in the cluster are subjected to the same basic environment which suggests that they either are stimulated at different rates or at different times.

The importance of this research lies in the fact that ovulation inhibition is the most prevalent contraceptive modality, and a better understanding of follicular dynamics may point to a new contraceptive approach.

Studies on Male Reproduction

One of the approaches designed to inhibit spermatogenesis in the male has been the utilization of various hormonal combinations. Studies in rats and rabbits have shown that spermatogenesis can be arrested through the use of testosterone in combination with estradiol implants. This research has now advanced to studies in male rhesus monkeys. Numerous dosage schedules are being tested. The dose of 100 μg testosterone plus 0.48 μg estradiol/kg/day led to the greatest suppression of sperm production. Sperm production was reduced by 96 to 98 percent without diminution in ejaculation volume. Although serum testosterone and estradiol values were elevated above control levels the differences were not significant. Additional treatment regimens have to be investigated to ascertain whether total spermatogenic suppression can be achieved without undesirable side effects.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title	:	Biological Testing Facility for the Evaluation of New Antifertility Drugs and Devices
Contract Number	:	NO1-HD-2-2746
Contractor	:	Mason Research Institute
Money Allocated	:	$844,057 (FY 72) 30 months; $116,436 (FY 73); $373,031 (FY 74); $463,828 (FY 75); $583,488 (FY 76); $725,005 (FY 77); $698,140 (FY 78); $845,501 (FY 79)

Objectives: The objective of this contract program is the establishment of a biological testing facility capable of broad spectrum evaluation of new drugs and devices and rapid exploitation of potential leads in the control of fertility. Sources of compounds for testing include contract synthetic programs supported by the CDB and numerous private, public and governmental laboratories throughout the world. The staff of the CDB reviews all compounds submitted for evaluation to determine the precise manner in which each drug will be studied.

Major Findings: The testing facility continues to evaluate new drugs, formulations and delivery systems in an efficient manner. The study of new analogs of LHRH has received emphasis during this period. Antagonists have been found which exhibit potent antiovulatory activity and agonists have been prepared which are extremely active as postcoital contraceptive agents.

A large number of long-acting androgens and estrus-suppressing steroids are being screened annually. The separation of classical estrogenic and postcoital antifertility activities of potent new steroidal estrogens has proven disappointing but it is now known that orally active estratrienes do not require the presence of a 17-ethynyl moiety. Nonetheless, the high activity observed in classical rodent assays is seldom expressed in rhesus monkeys using induction of withdrawal bleeding in ovariectomized animals as an endpoint.

The testing facility is developing a radioimmunoassay and drug monitoring capability which will permit more efficient evaluation of drug delivery systems and pharmacokinetics.

Significance to Biomedical Research and Program of the Institute: The heart of any drug development program is the rapid identification of biological activity and exploitation of promising leads. The ability of the CDB to evaluate compounds and devices in a uniform manner is of great importance to our contract synthetic program and the stated goals of the Branch.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Intrauterine Release of Estriol for Contraception
Contract No. : NO1-HD-3-2729
Contractor : Michael Reese Hospital
Money Allocated: $133,233 (FY 74); $172,289 (FY 75); $107,760 (FY 76); $143,154 (FY 78); $47,419 (FY 79); $42,210 (FY 80)

Objectives: The current objectives of this project are: (1) evaluation of the effects of an IUD releasing 12.5 µg/day of estriol in women; (2) evaluation of the long-term effect of intrauterine estriol on endometrial proliferation in baboons.

Major Findings: Evaluation of the effect of an IUD releasing 12.5 µg/day of estriol on endometrial histology and on endocrinology of the menstrual cycle in women scheduled to undergo elective hysterectomies for benign gynecologic diseases has just begun because of difficulties in obtaining liability insurance for the devices.

In order to determine the long-term effect of intrauterine administration of 25 µg/day of estriol on endometrial histology in baboons, devices have been introduced into the uteri of three baboons. Endometrial samples will continue to be obtained every six months and evaluated histologically.

Several experiments on ovariectomized, progesterone treated pregnant rats have been performed. The results demonstrate that estriol when injected as a bolus in the dose of either 0.1 or 1.0 µg does not induce implantation of blastocysts. Such implantation is however observed with the bolus injection of 0.1 µg of estradiol as well as with repeated q 3 x 24 hours injections of 0.1 µg of estriol or with a continuous release of estriol of 1.25 µg/24 hours. The results of these studies are in agreement with the data published by Clark et al (Endocrinology 100:91, 1977). The difference in biological effects of estradiol and estriol appears to be related to the nuclear retention time of receptor-estradiol vs receptor-estriol complexes. Repeated or continuous administration of estriol, which has brief nuclear retention time, results in the same biological effect as bolus administration of estradiol.

Significance to Biomedical Research and Program of the Institute: Development of new contraceptive methods is a stated goal of the Contraceptive Development Branch.

Proposed Course: This is expected to be a continuing contractual effort leading to important information on the pharmacodynamics of continuous administration of estriol.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Implantable Bio-Absorbable Capsules as Slow-Release Contraceptive Drug Delivery Systems
Contract No. : NO1-HD-3-2738
Contractor : Abcor, Inc.
Money Allocated: $254,856 (FY 73 for two years); $160,589 (FY 75); $129,079 (FY 76); $217,888 (FY 77); $283,560 (FY 78)

Objectives: The objective was to formulate biodegradable, sustained-release microcapsules containing progesterone. The system could also be utilized for the delivery of other contraceptive drugs where sustained long term delivery is desired.

Major Findings: Microencapsulation runs using the Wurster process were plagued by two major problems, agglomeration and nozzle plugging. Agglomeration was reduced by the removal of oversized and undersized particles from the bed by sieving and by close monitoring of the polymer addition rate. Nozzle plugging was solved by the combination of a higher solution addition rate, a lower solution concentration, and a larger atomizing nozzle.

Sieve analysis, scanning electron microscopy and in vitro release rate tests were used to characterize the microcapsules. In vivo degradation of the polymer over a one year period was also evaluated. The results indicate that complete degradation of the polymer approaches the one year target desired. The tissue reaction to the implant was generally acute or chronic inflammatory in nature. Fibrosis was apparent in some samples. Clusters of lymphocytes and occasional giant cells were reported. It should be noted that the implants were not sterile.

Approximately eight in vivo release studies were performed and involved three progesterone microcapsule batches. The effects of drug dose, injection volume and single vs. multiple sites were evaluated. In general, there was a burst of progesterone release with most doses in the first few weeks in all studies and blood levels progressively declined thereafter. Subsequently, there were periods of relative stability alternating with periods of fluctuation. It was not apparent that the levels were significantly above background. The in vivo studies on the norethindrone microcapsules were somewhat more encouraging; however, there were unexplained bursts at approximately 4 to 5 weeks.

Significance to Biomedical Research and Program of the Institute: Development of new drug delivery systems for contraceptive drugs is one of the goals of the Contraceptive Development Branch.

Proposed Course: This project did not receive additional support in FY 79; however, continued evaluation of the system by the Contraceptive Development Branch is planned and, if warranted, additional proposals will be sought in a competitive procurement.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : A Study of the Use of Biodegradable Polymers for the Sustained Delivery of Contraceptive Drugs
Contract No. : NO1-HD-3-2741
Contractor : Research Triangle Institute
Money Allocated: $298,347 (FY 73 for two years); $185,262 (FY 75); $224,082 (FY 76); $211,983 (FY 77); $210,081 (FY 78); $306,740 (FY 79); $258,688 (FY 80)

Objectives: To develop a biodegradabale polymer reservoir for subdermal delivery of a contraceptive steroid, e.g. norgestrel, for periods of one year or longer. The device is designed to provide constant release rate by diffusion control and to biodegrade after the drug is exhausted.

Major Findings: Pre-clinical studies necessary to obtain an IND for a 30 day clinical trial of a poly(ε-caprolactone)/levonorgestrel delivery system in 10 women were completed.

Higher and more constant rates of release of levonorgestrel from poly(ε-caprolactone) capsules were achieved by changing the dispersing agent. A slow decline in the release rate was shown to be the result of an increase in polymer crystallinity during degradation. In vivo (rat) release rates and blood levels were correlated.

Polymer degradation was determined in vivo (rat, rabbit) and in vitro under accelerated conditions (formic acid, 40°C). The decrease in molecular weight resulting from hydrolytic chain scission was associated with a slow increase in crystallinity and elastic modulus, and a decrease in permeability, ultimate tensile strength, and % elongation at break.

A 90-day toxicity study in rats and guinea pigs showed no evidence of local or systemic toxicity, and low molecular weight polymer powder did not evoke a significant inflammatory response. The rate and extent of excretion of ε-caprolactone monomer and ε-hydroxycaproic acid (a product of polymer hydrolysis) after subdermal injection in rats were determined. The efficacy of sterilization by γ-irradiation with 1.8 Mrads was demonstrated.

Certain copolymers of ε-caprolactone and δ-valerolactone were prepared and shown to possess superior mechanical properties and permeability.

Significance to Biomedical Research and Program of the Institute: Development of drug delivery systems for contraceptive drugs is one of the stated goals of the Contraceptive Development Branch.

Proposed Course: This is expected to be a continuing contractual effort leading to the development of improved contraceptive technology.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Development and Evaluation of a Biodegradable Drug Delivery System
Contract No. : NO1-HD-4-2802
Contractor : Arthur D. Little, Inc.
Money Allocated: $60,900 (FY 74); $195,625 (FY 75 for 2 years); $161,628 (FY 77); $144,764 (FY 78)

Objectives: The goal of this program is the development of implantable release systems that administer levonorgestral at a rate of 50 to 100 micrograms per day for approximately six months and that biodegrade within twelve months.

Major Findings: During the past year, depots were developed in the form of rods composed of a 50/50 mixture of drug and polypeptide. The polypeptides were copolymers of glutamic acid and ethyl glutamate. These copolymers appeared to be biocompatible, nonantigenic, and completely biodegradable. Studies involving radiolabeled copolymers revealed that the degradation product was glutamic acid, and this amino acid ultimately entered the metabolic pool.

The glutamic acid content of the copolymer determines its moisture sorption and biodegradation rates - two properties that influence levonorgestrerelease. A copolymer containing approximately 20 mole percent glutamic and exhibited the best balance between control of release and biodegradation.

Currently, three promising rod systems are on test in rabbits, rats and mice.

Significance to Biomedical Research and Program of the Institute: Development of drug delivery systems for contraceptive drugs is one of the stated goals of the Contraceptive Development Branch.

Proposed Course: This is expected to be a continuing contractual effort leading to the development of improved contraceptive technology.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Studies on Sperm Transport Through the Cervix
Contract No. : NO1-HD-4-2826
Contractor : University of Washington
Money Allocated: $53,955 (FY 74 for two years); $39,092 (FY 76); $48,199 (FY 77); $60,897 (FY 78)

Objectives: The objectives of this research are to explore the mechanisms of sperm penetration through the cervical mucus both in vitro and in vivo and to explore whether sperm penetration can be influenced by a variety of chemical approaches.

Major Findings: The biophysical properties of bovine and human cervical mucus have been evaluated by the non-invasive technique of laser light scattering spectroscopy. Although there are differences in chemical composition between bovine and human cervical mucus, their biophysical properties and biological functions are very similar. Both types of mucus can be described as an ensemble of entangled random-coiled mucins. The penetration of human spermatozoa into bovine cervical mucus is remarkably similar to their penetration into human cervical mucus. There is an increasing number of patients who have had excessive cone biopsies, cryotherapy or cautery and are incapable of producing sufficient cervical mucus to effect sperm transport. It is possible that estrus cow cervical mucus may be a suitable substitute in the clinical treatment of such patients. To avoid infection Co-60 radiation will be used to sterilize the cow cervical mucus at a dose which would not alter its molecular structure. The stability of the sterile estrus mucus stored at sub-zero temperatures has also been evaluated. By observing the ferning pattern and the penetration of human spermatozoa into these previously frozen specimens, and from the studies of laser light-scattering spectroscopy, it would appear that the preparations are stable.

Significance to Biomedical Research and Program of the Institute: Research dealing with sperm transport through the female reproductive tract is essential for the formulation of new methods of fertility regulation and is directly related to the mission of the Contraceptive Development Branch.

Proposed Course: The objectives of this project were met during FY 79.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis of Antifertility Compounds. Novel Compounds Structurally Related to Steroids.
Contract No. : NO1-HD-4-2846
Contractor : Oklahoma State University
Money Allocated: $17,575 (FY 74); $11,155 (FY 75); $39,971 (FY 76); $78,863 (FY 77 for eighteen months)

Objectives: The purpose of this project is to synthesize analogs of contraceptive steroids in which the B/C rings have been separated by a four-membered ring. These compounds will be tested for the antifertility activity.

Major Findings: Introduction of an angular methyl group by means of Michael addition of lithium dimethyl cuprate to a 1:1 mixture of the α,β-unsaturated ketone yielded exclusively a C-13 alpha oriented methyl isomer (cis fusion). Its stercochemistry was confirmed by a single crystal x-ray analysis. At present, two alternate synthic routes leading to cyclobuta-BC-estrone are under investigation. None of the cyclobuta-BC estrone homologs submitted for testing showed significant biological activity.

Significance to Biomedical Research and Program to the Institute: The work undertaken in this project is directly relevant to the purpose of the Contraceptive Development Branch to support the synthesis and evaluation of novel steroidal compounds for contraceptive utility.

Proposed Course: This is the terminal year for this contract.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Development of a Long-Acting Injectable Contraceptive
Contract Number : NO1-HD-5-2817
Contractor : Schering AG
Money Allocated : $1,211,168 (FY 75) 3 years; $120,000 (FY 78); $142,477 (FY 79)

Objectives: The purpose of this contract program is to acquire sufficient data to support the filing of a New Drug Application (NDA) on norethindrone enanthate, a long-acting progestational agent. This will require support of preclinical, clinical and long-term drug safety studies.

Major Findings: Data from two-year terminal sacrifices in two separate rat toxicity studies and from two-year interim sacrifices in dogs and monkeys has been submitted to the Food and Drug Administration in support of expanded Phase II clinical trials. Sponsor will review all clinical and preclinical drug safety studies at a September meeting of the Fertility and Maternal Health Drugs Advisory Committee to the FDA as requested by the Agency prior to approval to initiate Phase II clinical studies.

Metabolic studies and serum levels of norethindrone obtained during Phase I clinical trials will be completed within a few months.

Problems which have arisen in the development of a radioimmunoassay procedure for the enanthate ester are being resolved.

Significance to Biomedical Research and Program of the Institute: The development of a long-acting injectable contraceptive is directly related to the goals of the Contraceptive Development Branch.

Proposed Course: This is expected to be a continuing contractual effort encompassing completion of all preclinical and clinical requirements for the filing of a New Drug Application (NDA) and long-term drug safety studies in dogs and monkeys.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Detection of Ovulation by a Steroid Indicator
Contract Number : NO1-HD-5-2849
Contractor : The Abraham Lincoln School of Medicine
Money Allocated : $40,932 (FY 75), $41,670 (FY 76), $43,426 (FY 77)
$49,943 (FY 78), Approx. $40,190 (FY 79)

Objectives: The proposed study is designed to test the effectiveness of an indicator designed to ascertain the occurrence of ovulation in women. The indicator will detect semiquantitatively the postovulatory rise in either progesterone or a progesterone metabolite in saliva or pregnanediol-3-glucuronide in urine.

Major Findings: The feasibility of detecting ovulation in women by the use of an indicator strip that changes color in response to an increase in the concentration of pregnanediol glucuronide (PG) in urine has been investigated. Observations of an increase in the concentration of PG in urine parallel to the rise in serum progesterone after the preovulatory surge of LH support the concept. Further, it has been discovered that the concentration of PG in the first urine voided in the morning has the highest concentration of PG and reflects very rapidly and reliably the increase in progesterone secretion that accompanies ovulation.

The color change in the indicator strip will be based on uptake of fluorescein-conjugated antipregnanediol antibodies in excess of that required to bind PG in the urine sample. To this end fluorescein conjugation of the partially purified antiserum has been achieved without significant loss in binding activity. The indicator strip must have conjugated to it a derivative of pregnanediol that will bind the antiserum; a ∂-aminopropyltriethoxysilane derivative of silica gel on a plastic sheet has been shown to form an amine bond with a steroid hemisuccinate. For stability and specificity of conjugation a 3α-phosphate derivative of pregnanediol is preferred. A procedure for formation of such derivatives has been devised.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of the new female methods of contraception.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Contraceptive Development Studies for Males: Parenteral Testosterone Administration
Contract Number : NO1-HD-6-2815
Contractor : University of Washington
Money Allocated : $236,966 (FY 76 for two years); $185,835 (FY 77)

Objectives: This contract proposal is designed to evaluate the clinical safety, efficacy, and acceptability of intermittently administered testosterone enanthate as a single agent to produce reversible azoospermia in the human male. Such data would permit us to evaluate this concept of controlling male fertility in anticipation of the availability of new and more potent androgens.

Major Findings: Testosterone enanthate was administered to normal male adult volunteers, to determine whether or not an appropriate drug exposure schedule could induce azoospermia or marked oligospermia in close to 100% of the volunteers. Two main groups received either (1) 200 mg testosterone enanthate once weekly (I) or (2) 200 mg testosterone every two weeks (II).. Within 4 to 6 months 93% of the men in group I were azoospermic or oligospermic. Men in group I who became azoospermic were assigned to 2 subgroups by virtue of drug dosage changes. Group IA received 400 mg once monthly and group IB, 200 mg once monthly, in an attempt to maintain spermatogenic suppression while reducing the chemical burden on the body. Unfortunately there was a gradual return to almost normal spermatogenesis within 4-6 months in over 50% of the men. Recovery was assessed after 11 to 13 months drug exposure in all groups (I, IA, IB, and II) of men. By 12 to 16 months, 100% of the men recovered to pre-drug exposure levels. No drug-related adverse effect was noted on liver, kidney, bone marrow or electrolyte levels. Several volunteers noted mild, transient breast tenderness which disappeared either during drug exposure or during the recovery period. Serum LH and FSH levels were suppressed but there was no direct correlation between lack of suppression and failure to show azoospermia. Testosterone values increased 2-3 fold over control levels which lasted from 2 to 4 or 5 days after injection and returned to preinjection levels within 10 to 14 days. It was concluded that testosterone enanthate in its present formulation would have to be administered in doses of 200 mg every 10 to 14 days to be a reasonably effective, but safe and reversible chemical contraceptive agent for men.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of the new male methods of contraception.

Proposed Course: Completion

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title	:	Clinical Evaluation of Testosterone Enanthate for Induction of Reversible Azoospermia in the Human Male
Contract Number	:	NO1-HD-6-2817
Contractor	:	Professional Staff Association of Los Angeles
Money Allocated	:	$94,551 (FY 76), $102,900 (FY 77), $63,552 (FY 78), $24,198 (FY 78)

Objectives: This contract proposal is designed to evaluate the clinical safety, efficacy, and acceptability of intermittently administered testosterone enanthate as a single agent to produce reversible azoospermia in the human male. Such data would permit us to evaluate this concept of controlling male fertility in anticipation of the availability of new and more potent androgens.

Major Findings: In this study, testosterone enanthate (TE) was administered IM to 39 normal adult men (age 21-39) to assess its efficacy as a suppressor of spermatogenesis and to determine possible adverse effects. 17 subjects (Group A) received TE (200 mg/wk) for 16 to 20 weeks. 16/17 lowered sperm counts to less than 5 million/cc; 11/17 to less than 300,000; and 10/17 became azoospermic. Group B received TE (200/mg/2 wk.) In 10/22, sperm counts were less than 5 million/cc at 16 weeks; 9/22 were less than 300,000; and 5/22 were azoospermic. When those with sperm counts greater than 5 million/cc were switched at 16 weeks to weekly treatment (additional 3-16 weeks), 9/12 lowered sperm counts to less than 5 million/cc. Overall, 19/22 of Group B attained this level. Serum LH and FSH were decreased on both regimens. These effects were dose-related. Mean serum testosterone was elevated above baseline (+64%) at one week after injection (Group A), but remained at basal levels 2 weeks after TE injection in Group B. Testosterone binding protein concentrations were depressed by either weekly or bimonthly treatment; free testosterone levels were therefore higher than indicated by total serum testosterone. Serum estradiol levels parallel those of serum testosterone. Decreasing the frequency of TE (3 or 4 weeks) resulted in a rebound of FSH and LH above baseline and increased sperm counts. After discontinuing treatment, sperm counts and hormonal measurements returned to normal in all subjects. Modest increase in body weight, red cell mass, oiliness of skin and mild acne were seen in some subjects. Liver function tests, glucose tolerance, blood lipids and renal function were unchanged. Based on these data, testosterone enanthate demonstrates significant suppression of spermatogenesis. Further refinement of dose and/or delivery system, as well as investigation of combination hormonal regimens show promise for the development of a safe, effective male contraceptive agent.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of the new male methods of contraception.

Proposed Course: Completion

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Evaluation of Two Methods of Natural Family Planning
Contract No. : NO1-HD-6-2826
Contractor : Cedars-Sinai Medical Center
Money Allocated: $538,183 (FY 76); $353,000 (FY 77); $713,839 (FY 78)

Objectives: The objective of this clinical trial is to evaluate and compare the efficacy of two methods of natural family planning (ovulation and sympto-thermic) in a heterogeneous American population. The study will attempt to enroll 750 individuals into each one of the methods. It is anticipated that at the end of the three-year contract period there should be definitive data concerning the efficacy of the ovulation method and a reasonable partition of failures between method and subject categories. Furthermore, the study should provide information whether utilization of the mucus symptoms as a method of family planning can provide a level of efficacy comparable to the more complex sympto-thermal method. In its design the study will employ a monthly follow-up examination of menstrual cycles and pregnancy tests.

Major Findings:
A. The Sympto-thermal Method of NFP was clearly superior to the Ovulation Method in terms of use-effectiveness. This superiority was striking in all of the analyses presented: net rates, gross rates, and Pearl rates measured either from the beginning of training or from formal study entry showed at least a 2 to 1 advantage of ST over OM after 12 months of experience.

B. Randomization was successful in balancing the OM and ST groups with respect to various characteristics. Not only were the two groups in balance at the beginning of training but at time of formal entry into study as well.

C. The composite picture of participating couples attracted to the study was one of sexually active couples in their late twenties with a small family, high educational level, and predominantly white. Half of the group was Catholic, about half of the women were employed, and three-fourths were married. Accidental pregnancy would present few problems among these couples but they expressed a strong desire to refrain from sexual relations during fertile periods of the menstrual cycle.

D. Total dropout rates were high for both methods of NFP. Half of the women who started training dropped out during the training period and half of those who completed training and entered the study dropped out within the first year.

E. Voluntary termination of couples during training and in formal study was high. Lack of interest/dissatisfaction with method was the major factor involved in voluntary termination during the training period.

F. Recruiting difficulties constitute an important "finding" in this study mainly because they were not expected - certainly not to the degree experienced.

G. Means of couples completing training and entering the study were higher than means of training dropouts for age of wife, age of husband, number of previous pregnancies, number of living children, and years living with partner - and lower for number of monthly sexual relations.

Significance to Biomedical Research and Program of the Institute: This study should provide the first in-depth analysis of the efficacy of natural family planning methods and is directly related to the stated purpose of the Contraceptive Development Branch to support development of fertility regulating methods.

Proposed Course: Study was completed in FY 1979.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Isolation and Purification of Rat Androgen Binding Protein
Contract Number : NO1-HD-6-2842
Contractor : Baylor College of Medicine
Money Allocated : $47,379 (FY 76), $49,421 (FY 77), $53,631 (FY 78)

Objectives: The purpose of the proposed research is to isolate and purify androgen binding protein (ABP), to develop a radioimmunoassay for ABP, and to prepare radioimmunoassay kits for distribution to the scientific community. ABP is produced by the Sertoli cells upon stimulation by follicle stimulating hormone and its function is to transfer androgens to the tubular compartment of the testis and caput epididymis. Interference with the activity of ABP or its rate of synthesis could provide a means of selectively influencing the Sertoli cell response and, in turn, the regulation of spermatogenesis. A specific and sensitive ABP radioimmunoassay would be very useful for detecting early changes in ABP concentration in response to FSH or other hormonal treatments.

Major Findings: ABP was purified from batches of 1000 epididymides by affinity chromatography with Sepharose-diaminodipropylamine-dihydrotestosterone-17-hexanoate affinity resin. In some cases an additional chromatographic step was introduced to eliminate several species of contaminating proteins which resolved well from ABP on Sephadex G-200. For this purpose the concentrates from affinity chromatography eluates were subjected to further purification in DEAE-Sephadex and complete homogeneity was reported. By this procedure, the investigators have accumulated about 100 µg of highly purified ABP. The aminoacid composition and some physicochemical properties of the purified ABP were determined. The molecular weight of ABP determined by aminoacid composition was 105,500, similar to the value obtained by gel filtration which was 94,000. However, by SDS electrophoresis the MW was 47,000, indicating subunit a composition (dimer). ^{125}I-ABP has been recently prepared by iodination with ^{125}I-Bolton-Hunter reagent, with a specific activity of 100 µCi/µg. The latter will be used for assay of antibody titre directly by double antibody technique. However, as iodinated ABP was not available until recently, antibody titer was determined by steady state polyacrylamide electrophoresis and (^{3}H) DHT. The investigators have encountered problems with the purification procedure, related mainly to the very low yield obtained from the affinity chromatography step. However, no antibody against ABP has been successfully raised by these investigators.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of the new male methods of contraception.

Proposed Course: Terminated

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Potential Peptide Antagonists of LHRH as Ovulation Inhibitors
Contract No. : NO1-HD-6-2843
Contractor : University of Colorado Medical Center
Money Allocated: $59,348 (FY 76); $39,782 (FY 77); $45,839 (FY 78); $91,230 (FY 79)

Objectives: The goal of this project is to develop new highly potent antagonist analogs of LHRH.

Major Findings: During the past year approximately 25 new LHRH analogs have been synthesized and submitted for assay. Analogs containing an acylated D-amino acid in position one of LRH continue to yield inhibitors with highest known potency. A range of peptides has been synthesized to explore a variety of D-amino acids in position one, combined with several different acylating groups. The most potent of these yet tested is (acetyl-DAla^{1}, DPhe^{2}, DThi^{3,6})-LHRH (where Thi represents β-(2-thienyl)-alanine) which is effective in rats (anti-ovulating assay) at 50 µg. Further modifications of position one of antagonist analogs are being synthesized in the pursuit of higher potency inhibitors. Certain peptides have been tested which do not conform to the principle that analogs containing a D-amino acid in position two will always be antagonists. Certain such analogs containg additional modifications at the carboxyl end of the molecule possessed surprisingly high agonist activity and no antagonism. Some other analogs with restricted conformation or containing lipophilic groups have shown similar properties. The implications of these findings are being investigated and may lead to discovery of new types of "super agonists."

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of LHRH analogs for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Development of Formulations for Improving Efficacy of Orally Administered Natural Steroids
Contract No. : NO1-HD-6-2847
Contractor : Arthur D. Little, Inc.
Money Allocated: $217,978 (FY 76); $253,130 (FY 77); $272,544 (FY 78); $275,000 (FY 79)

Objectives: It is proposed to increase the bioavailability of orally administered estradiol. The formulations are based on mixed lipid micelles which are the product of enzymatic hydrolysis of fat plus components of bile.

Major Findings: The pharmocokinetics of estradiol has been characterized in both beagle dogs and Macaca fascicularis monkeys. Estradiol, administered i.v. (20% ethanol in saline) is rapidly distributed (V_D ≃ extracellular water) and rapidly metabolized ($t_{\frac{1}{2}}$ ≃ 10 min). The pattern of metabolites differs between the two species, yet one finds similar rates of elimination; i.e., "rapid" (40 min dogs, 3 hr monkeys) and "slow" (15-16 hr for both). The same pattern of metabolites are eliminated in both instances. Estradiol, administered p.o. in formulations, is slowly absorbed via the lymphatic system, bypassing both intestinal and liver metabolism and consequently, a greater percentage of the administered dose appears to enter the "slow" body pool ($t_{\frac{1}{2}}$ ≃ 15-16 hr). This "slow" body pool may represent the uptake and binding of estradiol into target organs. The size and turnover of the "slow" body pool is being evaluated and the importance of the "slow" body pool to therapeutic efficacy will be determined.

Significance to Biomedical Research and Program of the Institute: Increasing the bioavailability of orally administered natural steroids offers the potential of increased safety over the synthetic derivatives. Development of safe and efficacious contraceptives is the prime goal of the Contraceptive Development Branch.

Proposed Course: This is expected to be a continuing contractual effort leading to the development of improved contraceptive drugs.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis and Biological Testing of Luteolytic Prostaglandin Analogs
Contract No. : NO1-HD-7-2816
Contractor ; University of Chicago
Money Allocated: $137,214 (FY 77); $134,539 (FY 78); $150,000 (FY 79)

Objectives: The major objectives of this project are: 1) To synthesize 10, 10-difluoro-prostaglandin analogs for evaluation as luteolytic agents in the sheep and monkey; 2) Evaluate previously synthesized 16-fluoro-13-dehydro-prostaglandin analogs (see contract number NO1-HD-4-2801) for luteolytic activity and smooth muscle activity in the monkey.

Major Findings: The synthesis of 10,10-difluoro-13-dehydro-$PGF_{2\alpha}$ (as a 1:1 mixture with ent-15-epi-10,10-difluoro-13-dehydro-$PGF_{2\alpha}$) has been completed. Preliminary testing showed this material to be at least twice as active as $PGF_{2\alpha}$ in the hamster antifertility assay and to possess 20% and 13-15% of the activity of $PGF_{2\alpha}$ in the gerbil colon and hamster uterus assays, respectively. Preliminary data indicate similar activity in the ovarian sheep transplant.

Four compounds have been extensively studied in Macaca fascicularis. They are: 13-dehydro-$PGF_{2\alpha}$, ent-15-epi-13-dehydro-$PGF_{2\alpha}$, 16-fluoro-13-dehydro-$PGF_{2\alpha}$ (erythro form) and ent-15-epi-16-fluoro-13-dehydro-$PGF_{2\alpha}$. All of the compounds showed activity at the appropriate dose levels in the cycling monkey, that is, better than 90% suppression of circulating progesterone. However, in the pregnant animal only 13-dehydro-16-fluoro-$PGF_{2\alpha}$ was found to be effective in reducing circulating progesterone, yet ineffective in preventing pregnancy. These results suggest that circulating MCG is rendering the corpus luteum less susceptible to the action of the prostaglandin. Another factor that may be responsible for this failure is the lateness of the administration of the prostaglandin derivative at a time when placental progesterone secretion has already become important in this species, thereby protecting the fetus. Experiments are being planned using HCG treated nonpregnant animals. Examination of the animals for smooth muscle effects have not shown observable symptoms except in the prostaglandin $F_{2\alpha}$ controls.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of prostaglandins and related analogs for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Abeo Steroids as Contraceptive Agents
Contract No. : NO1-HD-7-2817
Contractor : Stanford Research Institute
Money Allocated: $156,538 (FY 77); $79,720 (FY 78)

Objectives: The objective of this contract is to combine serveral structural features [17β-ethyl, 11β-hydroxy and 9(10→19) abeo estratriene steroidal systems] in order to produce the optimal structural combination having high potency and the desirable separation of antifertility from estrogenic activity. Such compounds would be useful postcoital agents.

Major Findings: Previous structure activity studies revealed that modification of ethynylestradiol by introduction of C-11 substituents markedly affected uterotropic and postcoital antifertility activities. Based on these leads an efficient synthesis of 11-substituted-9(10→19)-abeo derivatives has been sought and finally achieved. Six of the target compounds 3,17β-dihydroxy-17α-ethynyl-9(10→19)-abeo-estra-1,3,5(10),9(19)-tetraen-11-one 3-methylether, 17α-ethynyl-9(10→19)-abeo-estra-1,3,5(10),9(19)-tetraen-3,17β-triol 3-methylether, 17β-ethyl-9(10→19)-abeo-estra-1,3,5(10),9(19)-tetraen-3,11β-diol 3-methylether, 17α-ethynyl-9(10→19)-abeo-estra-1,3,5(10)-tetraen-3,11β,17β-triol 17β-ethyl-9 (10→19)-abeo-estra-1,3,5(10),9(19)-tetraen-3,11β-diol and 17β-ethynyl-9(10→19)-abeo-estra-1,3,5(10),9(19)-tetraen-311β,17β-triol 3,11-dimethyl ether have been synthesized, characterized and submitted for estrogenic and postcoital antifertility assays. Contrary to what would have been expected on the basis of previous structure activity studies none of the compounds showed significant biological activity. Two non-target abeo derivatives 17α-ethynyl-9(10→19)-abeo-estra-1,3,5(10),9(19)-tetraen-3,11α,17β-triol 3-methyl ether and 3-hydroxy-9(10→19)-abeo-estra-1,3,5(10),9(19),11-pentaen-17-one were synthesized and characterized and tested for estrogenic and postcoital antifertility activity but no significant biological activity was observed. Unforeseen synthetic difficulties made it impractical to synthesize three of the original target compounds including a flat linear abeo triene version of the contraceptive triene steroid R-2323 as well as abeo-steroid with a highly strained double bond at the bridge head (C-11 position). As a result the synthesis of three alternative compounds are currently under investigation.

Significance to Biomedical Research and the Program of the Institute: The work to be undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of unusual modifications of steroids for contraceptive utility.

Proposed Course: The objectives of the contract were completed.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis and Testing of Androgenic Agents for Contraception
Contract No. : NO1-HD-7-2818
Contractor : Research Triangle Institute
Money Allocated: $96,479 (FY 77); $64,292 (FY 78)

Objectives: The objective of this project is to synthesize potent, orally active (non-17-alkylated) and long-lasting androgens as potential male contraceptive agents.

Major Findings: Sufficient quantities of the compounds 17β-hydroxy-7α,11β-dimethylestr-4-en-3-one have been synthesized to permit studies of its androgenic activity in rats. Two synthetic routes were developed. The better method starts with a readily available adrenosterone leading to the desired compound in a stereoselective manner. It has previously been shown to bind strongly to the androgen receptor from rat ventral prostate. In rats, it had an androgenic potency of 14-16 times that of testosterone after subcutaneous administration. Its effect on levator ani weight was approximately 30-32 times that of testosterone. This latter test is an indication of anabolic activity. Several analogs and intermediates are also being made available for testing. In particular, one intermediate, 11-methylene-3-methoxyestra-1,3,5(10)-trien-17-one, has been shown to be a potent uterotropic agent (45-95% relative to estrone in the immature rat). However, in contrast to the usual correlation of uterotropic and postcoital antifertility activity, this compound is ineffective as a postcoital antifertility agent. This unusual separation of activities is of considerable scientific interest and understanding of it could lead to development of effective postcoital antifertility agents.

Significance to Biomedical Research and the Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of steroidal androgens for contraceptive utility.

Proposed Course: The objectives of the contract were completed.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Development and Testing of New Biodegradable Drug Delivery Systems
Contract No. : NO1-HD-7-2824
Contractor : University of Utah
Money Allocated: $124,612 (FY 77); $219,750 (FY 78); $199,223 (FY 79)

Objectives: The goal of this research proposal is to synthesize and characterize biodegradable polymers of glutamic acid to which a progestin is covalently bonded. The polymer system should be capable of delivering drug by means of hydrolytic cleavage in vivo for 6-12 months.

Major Findings: Norethindrone has been successfully bonded to poly[N^5-(3-hydroxypropyl)-L-glutamine] via a 17-carbonate ester linkage (Compound XXb). Pilot studies involving bonding of norethindrone to poly[N^5-(3-hydroxyethyl)-L-glutamine] also via the 17-carbonate ester linkage (Compound XXa) also have been successful. Reaction of norethindrone-3-oxime with the free carboxyl groups of poly-(L-glutamic acid) to form the esterified compound (Compound XXII) has been completed. Compound XXb has been obtained as a powder in various particle sizes ranging from approximately 10μ to about 150μ. Methods have also been developed for controlled variation in the per cent of loading of norethindrone onto available reactive sites on poly[N^5-(3-hydroxypropyl)-L-glutamine].

In vitro studies on hydrolysis of low molecular weight carbonate esters (methyl, propyl, hexyl) of norethindrone shows that hydrolysis of the ester in solubilized molecules occurs slowly in aqueous buffer, but very rapidly in plasma. This strongly suggests that polymeric fragments attached to norethindrone are rapidly hydrolyzed once the molecule in solution comes into contact with enzymes found in plasma.

In vivo release studies on Compound XXb implanted subcutaneously in rats showed a somewhat steady, slowly declining, release pattern over the 256 days of the study. A preliminary study on Compound XXII implanted subcutaneously indicate that norethindrone is rapidly released from this polymer, i.e. 56 days for complete release.

Studies on the immunogenicity of the basic polymers and on Compound XXb indicate that these agents have a very low order of antigenicity.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the published purpose of the Contraceptive Development Branch to support research directed towards development of new methods of contraception.

Proposed Course: This is expected to be a continuing contractual effort leading to the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : New Biodegradable Drug Delivery Systems
Contract No. : NO1-HD-7-2825
Contractor : ALZA Corporation
Money Allocated: $206,289 (FY 77); $204,284 (FY 78); $455,411 (FY 79)

Objectives: The goal of this project is the development of a biodegradable, implantable delivery system that will deliver predictable, contraceptive amounts of synthetic progestins over a four to six month period.

Major Findings: The past year has seen action on many important tasks, including scaled-up synthesis (kilogram quantities) of CHRONOMER™, fabrication and quality assurance testing of 3000 norethindrone (NET) systems, development of quality assurance tests for NET systems (including *in vitro* release rate assays), purchase and testing of a larger mixer for scale-up of device production, completion of NET and commencement of norgestrel (Ng) functionality tests in baboons, completion of a six-week, subcutaneous irritation test of NET systems and placebos in baboons, important progress on Ng formulation selection (a reduction to three formulation candidates), completion of all test methodology except release rate determinations for ultimate Ng formulations, standardization and selection of an outside laboratory for NET and Ng assasys, and preliminary package development studies.

Current problems under investigation relate to evaluating the presence of extent of local tissue reaction in humans, developing adequate assays to ensure the purity of CHRONOMER™ synthesized by third parties, determining the optimal materials and design of packages that will protect the systems from hostile environmental changes, defining the final formulation and control tests for a six-month Ng system, and identifying a tool for subcutaneous injection of the systems.

Significance to Biomedical Research and Program of the Institute: Development of new drug delivery systems for contraceptive drugs is one of the goals of the Contraceptive Development Branch.

Proposed Course: This is expected to be a continuing contractual effort leading to the development of improved contraceptive technology.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Development and Testing of New Biodegradable Drug Delivery Systems
Contract No. : NO1-HD-7-2826
Contractor : SRI International
Money Allocated: $149,786 (FY 77); $237,555 (FY 78); $270,465 (FY 79)

Objectives: It is proposed to develop non-toxic biodegradable polymers capable of delivering a contraceptive agent with zero order kinetics over a six-month period. Additionally, drug release and polymer solubilization should take place concomitantly so that rate of drug release is controlled by the rate of polymer dissolution.

Major Findings: The investigation of activated polyesters has continued and considerable progress has been made in developing polyacetals as potentially useful bioerodible matrices.

Work with activated polyesters involved study of polymers based on diglycolic, perfluorosuccinic and α-ketoglutaric acids, each with trans cyclohexane dimethanol. Polymers based on diglycolic acid eroded too slowly to be of interest and perfluorosuccinic acid was shown to have biologic activity. Furthermore, both systems underwent bulk degradation with consequent deterioration of mechanical properties. Polymers based on α-ketoglutaric acid could not be prepared in linear form, but systems prepared by uncontrolled crosslinking exhibited promising erosion and drug release behavior. Because the degree of crosslinking could not be controlled, work with these polyesters has been discontinued.

Two approaches have been prepared with the polyacetals. In the first approach linear activated polyacetals are being synthesized and the interior of the matrix is being stabilized with basic buffers. In this way bulk degradation is prevented and the hydrolysis reaction is largely confined to the surface of the device.

In the second approach polyacetals are crosslinked. With these systems bulk degradation does not result in deterioration of mechanical properties and the combination of diffusional release and bulk degradation can yield acceptable drug release kinetics.

Significance to Biomedical Research and the Program of the Institute: The work undertaken in this project is directly relevant to the published purpose of the Contraceptive Development Branch to support research directed towards development of new methods of contraception.

Proposed Course: This is expected to be a continuing contractual effort leading to the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : The Synthesis and Testing of Polymeric Intravaginal Contraceptives
Contract No. : NO1-HD-7-2828
Contractor : SRI International
Money Allocated: $68,050 (FY 77); $28,871 (FY 78); $12,000 (FY 79)

Objectives: The objective of this project is to synthesize relatively large polymeric intravaginal contraceptives in order to decrease the absorption of the contraceptive agent across the vaginal barrier (by increasing their molecular weight), and thereby preventing systemic side effects as well as lengthening the period of potency.

Major Findings: During the past year, a polymeric sulfhydryl group reagent, N-(ethoxy polyethylene oxide) maleimide, and two polymeric disulfide group reagents, mercaptosuccinylated polyvinylmethylamine and copoly(vinylpyrrolidinone-vinyl thiol) were synthesized and tested. Sulfhydryl and disulfide group reagents are reported to interact with the spermatozoan membrane and immobilize sperm. Unfortunately, the polymeric versions of these reagents did not inhibit the motility of human spermatozoa.

Reducing the hydrogen ion concentration of the medium has been reported to have a spermicidal effect. Both polyvinylmethylamine hydrochloride and polyacrylic acid were found to lower the pH of semen and immobilize spermatozoa.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of new intravaginal contraceptives.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Development of a Radioimmunoassay for Rhesus Monkey Luteinizing Hormone
Contract Number : NO1-HD-7-2829
Contractor : University of Pittsburgh
Money Allocated : $66,331 (FY 77); $65,160 (FY 78); Approx. $66,331 (FY 79); $65,160 (FY 80)

Objectives: The aim of the program is to develop a radioimmunoassay for rhesus monkey luteinizing hormone (LH) and to provide sufficient reagents to prepare 200 kits (15,000 tubes per kit) for distribution to the scientific community.

Major Findings: Two partially purified rhesus monkey LH standards have been prepared both of which are about 10% pure. One of these, labeled WDP-X111-127-21, is well-characterized and has been used in the studies to develop the RIA kit. This preparation appears to be similar to plasma LH in the Leydig cell bioassay (parallelism in both assays and similar bio-to immunoassay ratios of activity). A second monkey LH standard, denominated WDP-XV-20, has been prepared in sufficient quantities to fulfill the needs of a kit. This preparation is not yet as well characterized as the first, but it has been prepared for vialing and subsequent distribution to selected laboratories for further evaluation.

110 rabbits have been immunized against LH or related hormones (57 against hCG, 36 against bLH, and 17 against oLH). When tested with ^{131}I-mLH, several would appear to be immediately useful for a kit if mLH were available in large enough quantities for routine, widespread radioiodination. Of the many combinations of hormone antiserum (anti-hCG, anti-oLH, anti-bLH) and radioiodinated hormone (oLH, rLH, hCG, hCG-β used, only 4 appear to be promising for use to measure serum LH levels in the monkey. The four are: (1) anti-hCG R20/^{131}rLH; (2) anti-bLH R79/^{131}I-hCG β; (3) anti-hCG R82/^{131} loLH; and (4) anti-hCG R91/^{131} I-rLH. The criteria of suitability include reasonable titers of antibody (1:18,000 or greater) and a sensitivity in the range required for detecting circulating LH levels in the monkey.

Significance to Biomedical Research and Program of the Institute: The work to be undertaken in this project is directly relevant to the published purpose of the Contraceptive Development Branch to acquire specific substances concerned with aspects of reproduction processes that are subject to experimental interventions.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Development of Orally Active Sustained Release Dosage Forms for Synthetic Steroids
Contract No. : NO1-HD-7-2830
Contractor : The University of Kentucky Research Foundation
Money Allocated: $101,500 (FY 77); $102,000 (FY 78); $150,000 (FY 79)

Objectives: It is proposed to develop a sustained release dosage form which will release norethindrone and ethynyl estradiol at such a rate that effective plasma levels of these synthetic steroids will be maintained for up to 24 hours.

Major Findings: Ethynyl estradiol and norethindrone have been formulated in sustained release tablets using FDA approved tablet excipients. Three different strength sustained release tablets were prepared for each steroid: 0.035 mg, 0.018 mg, and 0.009 mg for ethynyl estradiol, and 0.5 mg, 0.25 mg, and 0.125 mg for norethindrone. The dissolution profiles for the ethynyl estradiol tablets were identical and independent of the content of ethynyl estradiol. The same results were found for the norethindrone tablets.

In in vivo studies using beagle dogs it was found that ethynyl estradiol is subject to extensive first-pass metabolism and the bioavailability from the comparison of oral and IV administration is less than 6%. The results from administration of different doses indicate the first-pass metabolism of ethynyl estradiol is dose dependent and suggest the possibility of saturable metabolism.

Norethindrone was not metabolized as extensively as ethynyl estradiol on the first pass after oral administration and the availability was found to be 70-80% compared to IV administration. Furthermore, the area under the plasma level curve was found to be directly proportional to the dose of norethindrone indicating dose independent metabolism.

The results of this investigation demonstrate that contraceptive synthetic steroids can be formulated in sustained release tablets which result in lower peak blood levels.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the published purpose of the Contraceptive Development Branch to support research directed towards development of new methods of contraception.

Proposed Course: This is expeacted to be a continuing contractual effort leading to the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Development of Orally Active Dosage Forms for Steroids
Contract No. : NO1-HD-7-2831
Contractor : Southwest Research Institute
Money Allocated: $95,027 (FY 77); $104,104 (FY 78); $164,336 (FY 79)

Objectives: The aim of this program is to prepare sustained-release oral formulations of ethynyl estradiol and norethindrone, by incorporating these products in a matrix or microcapsule and to determine whether this dosage form will minimize daily peaks of the drugs and reduce body burdens while maintaining contraceptive efficacy and clinical acceptability.

Major Findings: Sustained-release microspheres of ethynyl estradiol (EE) and norethindrone (NET) dissolved in a mixture of mono-, di- and triglycerides have been prepared in the size range of 410 to 1000 microns using the Southwest Research Institute centrifugal encapsulation device. In vitro release-rate studies of these NET and EE containing microspheres, using synthetic gastrointestinal fluids, indicate a sustained release of the steroid over an eight-hour period.

Studies with intravenous administration of the NET and EE were carried out to examine disappearance rates and to serve as baselines for estimation of bioavailability. Oral administration of aqueous alcoholic solutions, aqueous solutions including a small amount of propylene glycol, tablets of commercial preparations, and suspensions of the various sustained-release formulations have been evaluated. As with the human, rapid conversion of ethynyl estradiol to conjugates, presumably sulfates, takes place. The kinetics of norethindrone in the baboon also seems to be very similar to that of the human. The sustained-release formulations gave prolonged plasma levels in vivo and an IND will be sought for clinical evaluation.

Significance to Biomedical Research and the Program of the Institute: The work undertaken in this project is directly relevant to the published purpose of the Contraceptive Development Branch to support research directed towards development of new methods of contraception.

Proposed Course: This is expected to be a continuing contractual effort leading to the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Development of Orally Active Dosage Forms for Steroids
Contract No. : NO1-HD-7-2833
Contractor : INTERx Research Corporation
Money Allocated: $125,738 (FY 77); $140,834 (FY 78)

Objectives: It was proposed to synthesize prodrugs of progesterone, 17β-estradiol, and testosterone which would reduce metabolism or conjugation during absorption or during the first-pass through the liver and, thereby, increase bioavailability.

Major Findings; Thiazolidines derived from the reaction of cysteine esters with 3-keto-4,5-unsaturated sex hormones, progesterone and testosterone, have been prepared and characterized. These previously unknown thiazolidines were obtained as either the 4,5- or 5,6-double bond isomers. In the progesterone case, the 20-ketone was also converted to a thiazolidine group. The two double bond isomers of these bis-thiazolidines were also isolated.

The bis-thiazolidines of progesterone were inactive, both orally and subcutaneously. In vivo studies with the radiolabeled derivatives showed that the derivatives were not absorbed. Apparently the bis-thiazolidines are not absorbed because they are so insoluble in water.

Orally, the 4,5-double bond isomer of the thiazolidine of testosterone which was more potent than the 5,6-double bond isomer, had 10-50% of the activity of methyl testosterone.

New, easily hydrolyzed 3-ethers of estradiol have also been prepared. These ethers have exhibited some activity both orally and subcutaneously.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the published purpose of the Contraceptive Development Branch to support research directed towards development of new methods of contraception.

Proposed Course: Since the activity of these prodrugs was not significantly better than the natural compounds, continuation of this project is not proposed.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title	:	Folliculogenesis in the Rhesus Monkey during the Menstrual Cycle
Contract No.	:	NO1-HD-7-2835
Contractor	:	George Washington University
Money Allocated	:	$45,999 (FY 77); $22,204, (FY 78); $52,454 (FY 79)

Objectives: The objective of this contract is to determine the distribution of preantral follicles in serially sectioned rhesus monkey ovaries. It is anticipated that some waves of follicular growth and atresia will be identified at specific times during the menstrual cycle.

Major Findings: Results indicate that a definite cyclic change occurs during the 28-day cycle. Preantral follicles with diameters of 100 μm are most abundant during the follicular phase, whereas during the luteal phase follicles 50 μm in diameter predominate. Another interesting observation is that preantral follicles tend to develop in clusters which are composed of follicles of various sizes. This observation seems unusual as the follicles within this group are subjected to the same basic environment which suggests that they either are stimulated at different times or grow at different rates.

Significance to Biomedical Research and Program of the Institute: Since the ovarian follicle is the target for contraceptives the knowledge of its natural history is important for appreciation of the possible mechanisms of action of contraceptives.

Proposed Course: The project was originally scheduled to be completed in FY 1979. However, the new projected completion date is FY 1981.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Development of Orally Active Dosage Forms for Steroids
Contract No. : NO1-HD-7-2849
Contractor : Battelle Memorial Institute
Money Allocated: $124,628 (FY 77); $8,334 (FY 78)

Objectives: This program was directed towards the development of orally active dosage forms which would eliminate the serum steroid "peak effect" seen with present day contraceptive pills. The dosage forms investigated, i.e. microcapsules containing either ethynyl estradiol (EE) or d-Norgestrel (d-NG), were designed to provide sustained release of the desired dose during the first 6-8 hours following oral administration.

Major Findings: The relative rates that the steroids ethynyl estradiol and d-Norgestrel are released in vitro from cellulose acetate butyrate (CAB) microcapsules or capsules coated with hydrogenated vegetable oils (prills) were modified. However, zero-order release kinetics was not achieved. In nearly all of the in vitro studies, the kinetics were first-order based on the linearity of the cumulative release vs. $_{t}1/2$ plots.

Many factors can effect the in vitro release properties of the microcapsules including the capsule diameter, wall thickness, properties of the test solution, and the initial steroid loading of the capsules. The first-order release mechanism may be directly related to the high solubility of these steroids in the polymer wall.

Difficulties with the RIAs for NET and EE in dog sera prevented proper analysis of the in vivo data obtained for this species. Also because of the variability of the results of the rat studies, it is believed that this species is unsuitable for use in GI tract steroid-release studies. This conclusion is supported by the high rat-to-rat variability, the large number of rates required per experimental point, and the limited volume of blood that can be taken from each rat.

Significance to Biomedical Research and Program of the Institute: Development of new contraceptive methods is a stated goal of the Contraceptive Development Branch.

Proposed Course: Due to difficulties in obtaining in vivo data plus the first-order in vitro release pattern, this project has not been continued.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title	:	Contraceptive Efficacy of Androgen-Estrogen Polydimethylsiloxane Implants in Rhesus Monkeys
Contract Number	:	NO1-HD-8-2805
Contractor	:	The Johns Hopkins University
Money Allocated	:	$70,937 (FY 78), $163,604 (FY 79), $96,217 (FY 80)

Objectives: The overall goal of this study is to design a reversible contraceptive technique for the human male which does not alter sexual behavior, accessory sex organ function or result in untoward side effects. The immediate objects of this proposal are twofold: (1) To define the most efficacious contraceptive formulation of testosterone and estradiol delivered via PDS subdermal implants to rhesus monkeys; and (2) to test the effect of this contraceptive formulation on fertility and erythropoietic system, blood clotting mechanisms and major organ function in rhesus monkeys.

Major Findings: Five rhesus monkeys have been assigned to each of three treatment groups to test the effect of testosterone and estradiol administered via subdermal Silastic implants on sperm production, seminal plasma volume and serum testosterone and estradiol concentration. Monkeys are kept on respective treatments for approximately 4 months and then changed to new treatments. Five control monkeys were included for identification of any periodic variation in spermatogenesis. As of now, the effect of 19 testosterone-estradiol dosages on the criteria outlined above have been tested. Dose levels have included 20, 30, 36, 60, 100 and 200 µg testosterone/kg/day with and without 0.12, 0.24, 0.48 and 0.72 µg estradiol/kg/day. During the past year the control monkeys once again showed annual fluctuation in sperm production. Sperm per ejaculate in control monkeys reached a high of 500×10^6 in October of 1978 and a low of 200×10^6 in May of 1979. Sperm production followed a similar periodic fluctuation in animals treated with testosterone alone. By contrast, this seasonal variation was eliminated in monkeys receiving several testosterone-estradiol combinations. The dose of 100 µg testosterone plus 0.48 µg estradiol/kg/day has led to the greatest suppression of sperm production. Sperm per ejaculate in monkeys varied from 2-4% of the controls with several animals being azoospermic. This dose caused no significant difference in ejaculate volume. Although serum estradiol and testosterone concentrations were elevated above control levels the difference was not significant. Presently the effect of 100 µg testosterone/kg/day, with and without 0.36 and 0.96 µg estradiol/kg/day is being explored.

Significance to Biomedical Research and Program of the Institute: Development of new techniques for the control of fertility in the male is one of the stated goals of the program.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis of Postovulatory Prostanoid Antifertility Agents
Contract No. : NO1-HD-8-2808
Contractor : University of Washington
Money Allocated: $50,039 (FY 78); $51,887 (FY 79)

Objectives: The major objectives of this project are to synthesize prostanoids which will incorporate in a single molecule those structural modifications which have independently led to increased luteolytic potency and selectivity (reduced smooth muscle activity).

Major Findings: Past studies had uncovered a number of structural strategies for improving the luteolytic specificity of prostaglandins: 1) 17,17-methylation, 2) saturation of the 13,14-olefinic linkage, 3) 8,12-epimerization, 4) 18,18,20-methylation, and 5) the introduction of a perfluoroalkyl terminated ω side-chain. Although structural variations 1), 2), and 3) each produce at least a 10-fold improvement in luteolytic specificity with no loss in potency (typically a gain of 2-5 times) relative to $PGF_{2\alpha}$, in combination they do not provide further improvement in specificity. Therefore attention was focused on combining strategies 4) and 2) or 3) and on further studies of perfluoroalkyl terminated prostaglandins (some of which display improved specificity, by factors of 8-30.), and on the development of routes to bis-trifluoromethylated prostaglandins. It is anticipated that bis-trifluoromethylated prostaglandins will combine, in an additive manner, the beneficial effect of gem-dimethylation (a conformational effect) and fluorination (an electronic effect).

Complete bioassay data for the fluorinated omega side chains (Rω) indicate that the $PGF_{2\alpha}$ analog where $R\omega = C_4F_9CH_2CH_2-$ (potency is 160% of $PGF_{2\alpha}$, specificity factor equals 11) is the best candidate for further study and therefore synthesis of the corresponding 8,12-epi-PGE_2 analog is in progress.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of prostaglandins and related analogs for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis and Biological Evaluation of Luteolytic Agents
Contract No. : NO1-HD-8-2809
Contractor : University of Pittsburgh
Money Allocated: $99,740 (FY 78); $71,812 (FY 79)

Objectives: The objectives of this project are a) to synthesize 100 mg of optically active (+)-12α-fluoro-$PGF_{2\alpha}$ and to determine its luteolytic and uterine contracting activity in the monkey and b) to synthesize several fluoroprostaglandins substituted at the 10-, 12-, and 14-positions and to determine their antifertility effects in hamsters as well as their oxytocic and smooth muscle activities.

Major Findings: During the past year the total syntheses of (+)-10β-fluoro-$PGF_{2\alpha}$ methyl ester (1), (+)-15-epi-10β-fluoro$PGF_{2\alpha}$ methyl ester (2), (+)-(13Z)-14-fluoro$PGF_{2\alpha}$ methyl ester (3), (+)-(13Z)-15-epi-14-difluoro$PGF_{2\alpha}$ methyl ester (4), (+)-(13E)-14-fluoro$PGF_{2\alpha}$ methyl ester (5), (+)-(13E)-15-epi-14-fluoro$PGF_{2\alpha}$ methyl ester (6), and (+)-12-epi-12-fluoro$PGF_{2\alpha}$ methyl ester (7) were completed. The synthesis of 100 mg of (+)-12α-fluoro-$PGF_{2\alpha}$ was completed. The biological results continue to demonstrate that replacement of a proton on the prostaglandin skeleton by a fluorine atom results in suppression of smooth muscle stimulating activity while at the same time maintaining "luteolytic" potency. Thus, 5 was four times as effective as $PGF_{2\alpha}$ in the hamster while possessing 0.4-1.25 times the oxytocic activity of $PGF_{2\alpha}$ and 0.15-0.26 times the smooth muscle activity of $PGF_{2\alpha}$. [The corresponding 15-epi analog (6) was inactive]. (+)-10β-fluoro$PGF_{2\alpha}$ methyl ester (1) and (+)-15-epi-10β-fluoro$PGF_{2\alpha}$ methyl ester (2) were both effective in the hamster (25 μg and <50 μg respectively). Compound 1 possessed approximately 0.28-0.38 and 0.03-0.04 times the potency of $PGF_{2\alpha}$ in hamster uterine and gerbil colon strips, respectively, while the results with 2 were: 0.01 (hamster) and 0.02 (gerbil colon) relative to $PGF_{2\alpha}$. (+)-12-epi-12-fluoro$PGF_{2\alpha}$ was much less active than (+)-12α-fluoro$PGF_{2\alpha}$.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of prostaglandins and related analogs for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Inhibition of Progesterone Biosynthesis and Antiprogestational Agents
Contract No. : NO1-HD-8-2810
Contractor : SRI International
Money Allocated : $165,813 (FY 78); $143,239 (FY 79)

Objectives: The objective of this project is to synthesize a) inhibitors of progesterone biosynthesis, (e.g., inhibitors of 3β-hydroxysteroid dehydrogenase and Δ^5-3-ketosteroid isomerase) and b) competitive inhibitors of progesterone as represented by certain ring-D modified steroids.

Major Findings: With regard to the synthesis of 3β-dehydrogenase-Δ^5-isomerase inhibitors, 4,4-dimethyl-17β-hydroxy-17α-ethynyl androst-5-en-[2,3-d]isoxazole was prepared, and an enzyme inhibiton assay for this compound is in progress. The synthesis of two other target compounds, 17β-acetamido-4,4-dimethyl-androst-5-en[2,3-d]isoxazole and 4,4-dimethylpregn-5-en[2,3-d]isoxazole are well underway.

Based on previous structure activity studies and computer generated model studies six 15,16-seco and one C-homo pregnane derivatives were selected for synthesis as potential progesterone antagonists. The synthesis of the target seco compounds depends on the key intermediate 3-hydroxy-15,16-seco-estra-1,3,5(10)-trien-17-one 3-methyl ether and a good deal of the first nine months of the contract has been spent on developing an efficient route to obtain enough starting material to pursue the target compounds. The synthesis of three of the target seco compounds and the C-homo analog are currently underway. The in vitro and in vivo biological assays will be initiated as target compounds are available.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of new methods of contraception.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis and Testing of Antiovulatory and Postovulatory Agents
Contract No. : NO1-HD-8-2811
Contractor : Research Triangle Institute
Money Allocated: $152,781 (FY 78); $163,280 (FY 79)

Objectives: The objective of this contract is to synthesize potent aromatase inhibitors in order to block implantation, and to develop compounds which may be antiprogestational agents, possibly through inhibition on progesterone biosynthesis. These compounds will be tested for antifertility activity.

Major Findings: Synthetic routes have been developed for the formation of 7α-methyl substituted analogs of testolactone. In vitro tests have now shown that testolactone has extremely poor ability to inhibit aromatization by placental microsomes. Introduction of the 7α-methyl group does increase aromatase inhibition activity, but the compounds are still relatively weak inhibitors. To date, none of the aromatase inhibitors tested has shown significant activity as a postcoital antifertility agent in the hamster. Therefore, modifications of the basic testolactone structure are being synthesized. It appears that the development of a useful aromatase inhibitor will require a compound with an inherently greater affinity for the enzyme coupled with a long biological half-life. A mixed in vivo/in vitro test for aromatase inhibition has been developed in which the compound is administered to pregnant animals. At intervals after administration, ovaries are removed and their aromatase activity is measured. This test offers promise as a rapid means of determining the duration of aromatase inhibition as well as the potency of the compound.

Significance to Biomedical Research and the Program of the Institute: The work to be undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of usual modifications of steroids for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis and Testing of Progesterone Analogues of Novel Structures with Potential Antifertility Activities
Contract No. : NO1-HD-8-2812
Contractor : Laval University
Money Allocated: $74,715 (FY 78); $64,784 (FY 79)

Objectives: The objective of this contract is to synthesize derivatives of 17α-hydroxymethylprogesterone and 11α-substituted progesterone analogues in view of the potentiating effect of 17-alpha substituents and the influence of substitution at C-11 on progestational activity. These compounds will be tested for antifertility activity.

Major Findings: A new method of 17-hydroxymethylation of saturated 20-keto steroids, involving formylation, in high yield, of lithium 17,20-enolates, prepared from enol acetates, was developed. Thus, the synthesis of 17-acetoxymethylprogesterone has been simplified and improved.. The 11-methylated steroids were prepared by the reaction of 3,20-dioxygenated 11-bromo 12-ketones with lithium dimethylcuprate and by the treatment of the reaction product with methyl iodide. The reactions were carried out both in the A/B-trans and in the A/B-cis series, the starting materials being prepared respectively from the degradation product of hecogenin and from desoxycholic acid. In the A/B-trans series only the 11α-methyl adduct was obtained, in the A/B-cis series both 11-epimers were isolated, the 11α-methyl derivative predominating. When the 11-bromo 12-ketones are treated with lithium dimethylcuprate and the reaction mixture with formaldehyde, the 11α-hydroxymethyl 12-ketones are formed; they are accompanied by 11-methylated products, originating from the direct reaction of lithium dimethylcuprate with the bromo ketone. The 11α-hydroxymethylation of 12-ketones was achieved without concomitant formation of 11-methylated products, by converting the 3,20-dioxygenated 12-ketone to its p-tosylhydrazone, by treating the latter with n-butyllithium, and by subjecting the reaction product to the action of formaldehyde. Reduction with sodium borohydride leads to the 11α-hydroxymethylpregnane-3,20-diol.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of novel steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Specific Immunization Against FSH as a Male Contraceptive
Contract No. : NO1-HD-8-2814
Contractor : University of Colorado Medical Center
Money Allocated: $63,744 (FY 78); $69,268 (FY 79)

Objectives: The objective of this project is to produce specific anti-FSH sera to block spermatogenesis without effect on secondary sex characteristics and thus to develop an immunologically based male contraceptive.

Major Findings: To prevent undesirable side effects due to cross-reaction of the antibodies with LH and TSH, which would occur if the entire hFSH or β-chain were used as antigen, immunizations are being done with peptides comprising unique areas of the FSH sequence where no homology exists with LH and TSH. These areas are at residues 39-50, 67-75 and 95-118 of the hFSH β-chain (Saxena sequence numbering). Peptides containing all these unique areas have been synthesized and various antigens prepared from them. Immunizations of male rabbits with these antigens reliably yielded antisera against the peptides, but none of these so far have shown significant cross-reaction with the intact hFSH molecule. Synthetic peptides constituting residues 95-110 and 95-118 of hFSH-β reacted with a Parlow antiserum raised with the reduced hFSH β-chain, indicating that this region of the hormone may be an important antigenic site. Present work is directed toward synthesis of peptides encompassing larger areas of the FSH β-chain, conformationally restricted peptides (containing disulfide links introduced by cystine residues) and development of a new technique for synthesis of peptide antigens without the necessity of conjugation of the peptide to a large molecule carrier.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the development of a specific immune-based male contraceptive.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis and Testing of Novel Steroids with Potential Male Contraceptive Activity
Contract No. : NO1-HD-8-2815
Contractor : Laval University
Money Allocated: $33,812 (FY 78); $37,178 (FY 79)

Objectives: The objective of this contract is to synthesize 11-aza-5α-dihydrotestosterone analogs which will lead to the study of a distinctly different 11-hetero steroid. The importance of the 11-position in governing both estrogenicity and effects on gonadotrophin is now apparent. These compounds will be tested for the male contraceptive activity.

Major Findings: In view of the synthesis of 4,5α-dihydro-11-azatestosterone and of some of its derivatives, 3β-acetoxy-5α-pregn-16-ene-12,20-dione, the degradation product of hecogenin acetate has been transformed into 11-aza-5α-pregnane-3β,20β-diol, in a multistep synthesis, essentially according to procedures already reported. The starting material is first transformed into 3β,20β-dihydroxy-5α-pregn-9-en-12-one which is converted by ozonolysis and by subsequent reaction with hydrogen peroxide to 3β,20β-dihydroxy-9-oxo-9,12-seco-11-nor-5α-pregnanoic acid[1]. Ammonolysis leads to an 8,9-unsaturated lactam, which is reduced to the unsaturated amine, which in turn is reduced via the imminium chloride to the saturated amine. The sequence of reactions from the unsaturated lactam to 11-aza-5α-pregnane-3β-diol was improved and a first batch of 3.8 g of that product was obtained. The transformation of the 11-aza pregnanediol into its tris-trifluoracetyl derivative were achieved in over 80% yield, subsequently the protecting groups of the alcohols were selectively hydrolyzed and were oxidized with Jones' reagent. Thus the N-fluoroacetyl derivative of 11-aza-5α-pregnane-3,20-dione was obtained in very satisfactory yields. After mono-ketalization (with methanol) in position 3 - a reaction already investigated - the resulting derivative has to be hydroxylated in position 17 and the side chain of the product to be degraded. It has been also established that the protecting group of the amino function in position 11 could be removed without difficulty.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of novel steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis and Testing of Male Contraceptive Agents
Contract No. : NO1-HD-8-2816
Contractor : Stanford Research Institute International
Money Allocated: $42,271 (FY-78)

Objectives: The purpose of this project is to prepare potent orally active new androgens that will act on spermatogenesis by inhibiting gonadotrophin and maintain libido and secondary sex characteristics because of their great androgenic potency. These compounds will be tested for the male contraceptive activity.

Major Findings: The key intermediate, 11β-hydroxy-7α-methylestrone 3-acetate has been readily obtained by reaction of 7α-methylestrone acetate with ceric ammonium nitrate and subsequent removal of 9α-hydroxyl group followed by reductive hydrolysis of 11β-nitrate ester. This method turns out to be a unique procedure to introduce 11-substituents without too much difficulty starting from 7α-methyl estrone. Conversion of the above compound to the 3-methyl ether followed by Birch reduction led to 11β-hydroxy-7α-methyl-19-nortosterone. It was submitted for biological assay. 11β-Methoxy anolog will be available to us in the very near future for biological evaluation.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of novel steroidal compounds for contraceptive utility.

Proposed Course: This is the terminal year for this contract.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title	:	Synthesis and Testing of Male Contraceptive Agents
Contract No.	:	NO1-HD-8-2817
Contractor	:	SRI International
Money Allocated	:	$22,450 (FY 78); $23,805 (FY 79)

Objectives: The objectives of this project are to synthesize potential antispermatogenic agents. The compounds to be synthesized are analogs of 4-(p-cholorophenylthio)butanol which was found to have antispermatogenic properties in rats and beagles during the course of toxicological examination.

Major Findings: The lead compound, 4-p-chlorophenylthiobutanol, was found to be toxic at the antispermatogenic dose (500 mg/kg x 5 days). Even at 200 mg/kg the animals lost weight. Thus effort was focused upon trying to separate the two effects, if possible. Shortening the chain (3-p-chlorophenylthiopropanol) reduced activity as did replacing the chloro group by a t-butyl group. Other modifications designed to reduce toxicity are in progress.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards the development of male contraceptive agents.

Proposed Course: This is expected to be a continuing contractual effort leading towards the development of a male contraceptive and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis and Testing of Analogs of LHRH as Ovulation Inhibitors
Contract No. : NO1-HD-8-2819
Contractor : Tulane University
Money Allocated: $156,691 (FY 78); $151,986 (FY 79)

Objectives: The objective of this project is to synthesize and biologically evaluate analogs of LHRH (including dimers and trimers) modified at various amino acid residues in order to produce very potent and long-acting antiovulatory agents.

Major Findings: Combining previous observations on the importance of D-Phe in position 2 and acetyl amino acids in position 1 for producing high inhibitory activity, [D-Ac-Phe^{1}, D-p-Cl-Phe^{2}, D-Trp^{3,6}] LHRH was synthesized and found to be the most potent inhibitor to date: complete blockage of ovulation in the rat at 31μg, s.c.

Inhibitory analogs of LHRH were synthesized with D-Lys in place of Gly in position 6 of the chain. The free ε-amino group on the side-chain was then used as a dimerization point for reaction with a variety of aliphatic, aromatic, and alicyclic dicarboxylic acid active esters. In most instances, the dimeric compounds were considerably more active in the standard rat blockage of ovulation assay, the most effective being active at 125 μg per animal. Attempts to prepare similar dimers via the aromatic amino group of p-NH_2-D-Phe incorporated in the same position were unsuccessful due to lack of reactivity. Analogs containing N$^{\phi}$-Z-Ala-(p-NH_2-D-Phe) were, therefore, synthesized using α-Boc,N$^{\phi}$-NPS-(p-NH_2-D-Phe) from which the NPS group could be removed selectively allowing DCC-mediated coupling of Z-Ala to the free amino group. HF cleavage gave a peptide with a much more reactive α-amino group attached to the aromatic side-chain. Although these monomers were much more active than their D-Lys counterparts, dimerization gave peptides with only slightly increased inhibitory potencies.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of new contraceptive agents.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Peptide Antagonists of LHRH as Ovulation Inhibitors
Contract No. : NO1-HD-8-2820
Contractor : University of Texas at Austin
Money Allocated: $196,973 (FY 78); $212,122 (FY 79)

Objectives: The objective of this project is to synthesize and biologically evaluate analogs of LHRH (particularly changes in the 1- and 3- positions of [AA^1,D-Phe^2,AA^3,D-Trp^6]LHRH) in order to obtain very potent and long-acting antiovulatory agents.

Major Findings: Potent antiovulatory activity has been observed for the first time in analogs having a dipeptide in position 1, which is equivalent to an undecapeptide and focuses new attention on analogs have more than ten amino acids. The [Glu-Pro]^1 analog completely inhibited ovulation at 200 µg/rat and partially inhibited at 100 µg.

To date, the following guidelines have emerged from the search for antiovulatory peptides of increasing potency: (1) [residue^1, D-Phe^2, D-Trp^3, D-Trp^6] is a preferred substitution; (2) [residue]^1 may have the L- or D- configuration, and the configuration of the most potent [isomer]^1 depends on the residue as for example: D<Glu (200 µg)>L-Glu (750 µg) and Ac-Pro (200 µg)>Ac-D-Pro (750 µg); (3) some polar character is required as N-Ac-Thr>Ac-Pro>Cpc; (4) an N-protected residue appears necessary as Ac-Pro>Pro and Ac>H; (5) a (dipeptide)^1 can be accomodated at the receptor as (<Glu-Pro)^1 (200 µg)>(<Glu-Gly)^1 (750 µg)>(Gly-Pro)^1. The most potent analogs in vitro were [AcPro^1,DPhe^2,DTrp^{3,6}]-LH-RH, [AcHyp^1,DPhe^2, DTrp^{3,6}]-LH-RH, [(L<Glu-Pro)]^1.DPhe^2,DTrp^{3,6}]LH-RH and [D<Glu^1,DPhe^2,DTrp^3,DPhe^6]LH-RH. All of these analogs almost completely inhibited the LH-RH-LH response at 30 ng/ml medium and the concomitant LH-RH-FSH response was inhibited by 50% or greater. Also, these analogs inhibited the acute LH response to exogenous LH-RH in adult male rats at either a 30:1 or 100:1 ratio of the analog/LH-RH. At an analog/LH-RH ratio of 333:1, the above AcPro^1 analog and [L<Glu-Pro]^1 analog inhibited the acute LH response to exogenous LH-RH in adult male chimpanzees. Using the above 4 systems for evaluating the activity of a larger number of analogs, only a partial correlation betwwen the in vitro and in vivo assays has been observed. The most potent inhibitors of ovulation were always very potent in vitro, but other analogs having identical in vitro activities have had little or no antiovulatory activity, even at substantially higher dosages. Although these results could be interpreted that these inhibitors may act at dual sites, i.e., pituitary and ovary, only 1 of 8 analogs at a high dosage in vitro inhibited the binding of labelled LH to the gonadal LH receptor (performed by Leo Reichert). However, the possibility of action at the ovary by direct binding of the analog or by some post receptor mediated mechanism still exists.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of new contraceptive agents.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title	:	Peptide Antagonists of LRF as Ovulation Inhibitors
Contract Number	:	NO1-HD-8-2821
Contractor	:	Salk Institute for Biological Studies
Money Allocated	:	$173,715 (FY 78); $183,276 (FY 79)

Objectives: The objective of this project is to synthesize and biologically evaluate analogs of LHRH (taking advantage of the potent antagonist, [D-p-Glu^1, D-Phe^2,D-Trp^{3,6}] LRF already developed at Salk) containing modifications at positions 1-, 2-, 3-, 6-, 7- and 10 in order to obtain very potent and long-acting antiovulatory agents.

Major Findings: Systematic modifications at positions one, two, three, five, six and seven were introduced. These included a large number of acylated and non-acylated D-amino acid in position one, D-phenylalanine or D-tryosine at position two, D-tryptophane at position three, tyrosine or p-Cl-phenylalanine at position five, D-tryptophane and (imBzl)-D-His at position six and leucine and NαMe leucine at position 7. All potential LRF antagonists were tested in vitro for their abilities to inhibit the LRF mediated secretion of LH by primary anterior pituitary cell cultures. Potencies as IDR_{50} are expressed as the molar ratio (antagonist/LRF) required to reduce the amounts of LH secreted by 50%. Whereas the original standard, [D-pGlu^1,D-Phe^2,D-Trp^{3,6}] LRF had an IDR_{50} of 3/1 in the in vitro assay, a series of new analogs ([Ac-dehydro-D,L-Pro^1,D-Phe^2,D-Trp^{3,6}] -LRF, [dehydro-D,L-Pro^1,D-Phe^2,D-Trp^{3,6},pCl-Phe^5,NαMeLeu^7]-LRF and [Ac-D-Phe^1, D-Phe2,D-Trp^{3,6},NαMeLeu^7]-LRF) have an IDR_{50} of 0.3 in the same assay. This represents a ten-fold increase in potency. The abilities of analogs to compete with [^{3}H-Pro^9]-LRF or [D-pGlu^1,D-Phe^2,D-Trp^{3,6},^{3}H-Pro^9]-LRF for binding to pituitary membrane homogenates constitutes the basis of a "radioreceptor" assay. It has been observed that LRF antagonists have in general higher membrane binding affinities than would have been anticipated from their IDR_{50} values determined from the peptides' antagonism of LRF-mediated secretion of LH in vitro. The mechanisms responsible for this phenomenon are under study. Several of the antagonists most potent in vitro are also powerful inhibitors of ovulation in freely-cycling rats. [Ac-dehydro-D,L-Pro^1,D-Phe^2,D-Trp^{3,6}]-LRF blocks ovulation by 100% at a single dose of 100 µg per rat. It has also been shown that some antagonists are active postcoitally.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of new contraceptive agents.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Peptide Antagonists of LHRH as Ovulation Inhibitors
Contract No. : NO1-HD-8-2822
Contractor : University of Louisville Foundation, Inc.
Money Allocated: $118,956 (FY 78); $101,306 (FY 79)

Objectives: The objective of this project is to synthesize analogs of LHRH which contain a hydrolytically resistant non-toxic thiomethylene linkage as an amide replacement in strategic locations in order to eventually obtain very potent and long-acting antiovulatory agents.

Major Findings: While polypeptide hormones have often been modified by amino acid additions, deletions and substitutions, there has been less attention directed towards modifications of the amide backbone. Yet it is precisely this backbone that is most vulnerable to enzymatic breakdown and, as a result, loss of biological potency. The approach in this contract has involved the use of amide bond replacements, starting first with the thiomethylene ether ($-CH_2S-$), (Ψ), as a replacement for the amide function (-CONH-). Among virtues of this link are 1) synthetic accessibility from amino acid precursors, 2) retention of chirality at adjacent α-carbons, and 3)steric compatibility with the amide linkage.

The first members of the pseudopeptide class of LH-RH analogs synthesized have involved replacement at position 6-7 of LH-RH, [Gly Ψ $Leu^{6,7}$]LH-RH, and of a potent antagonist [D-$pGlu^1$, D-Phe^2, D-Trp^3, Gly Ψ $Leu^{6,7}$]LH-RH. Both of these compounds had surprisingly low activity, which suggests that the previously determined increase in activity by D-substitutions at the 6 position may be due to enhanced conformational rigidity rather than by increased enzymatic resistance. Synthetic routes to other members of the pseudodipeptides have also been elaborated during the first year; thus, the incorporation of more complex pseudopeptides at 6,7 such as D-Phe Ψ Leu (currently in progress) should provide further clarification of the dual factors of peptide conformation and peptide stability . The synthesis of analogs with pseudopeptide substitutions at the N- and C-termini and several other positions are currently underway. Finally, modifications of the pseudopeptide linkage itself, designed to provide the greater rigidity found in the quasi-planar amide bond, are also in progress.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of new contraceptive agents.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthetic Chemical Facility
Contract No. : NO1-HD-8-2823
Contractor : Southwest Foundation for Research and Education
Money Allocated: $147,323 (FY 78); $156,620 (FY 79); $166,014 (FY 80)

Objective: The purpose of this project is to have this synthetic chemical facility synthesize rapidly, laboratory scale quantities of specific compounds required by the CDB for contraceptive investigation.

Major Findings: During the first year of this contract a total of thirteen compounds have been synthesized, including steroidal and nonsteroidal compounds. These compounds can be classified into the following five categories: (1) novel compounds; (2) known but commercially unavailable compounds; (3) chemical intermediates in large quantities (e.g., 100 grams) in order to assist other ongoing synthetic projects; (4) radiolabeled synthesis including stable isotopes such as carbon-13; and (5) resolution of the racemates into the corresponding optical antipode. Several of these required large batch, and multi-step synthesis to obtain the desired product. For example, fifty-one grams of 3-methoxy-9(10→19) abeo-estra-1,3,5(10),9(19)-tetraene-11,17-dione were obtained in 27% overall yield starting from Δ^1-adrenosterone. A large-batch synthesis of 3,9-dimethoxy-6aα,6bβ,12aβ-tetrahydrodibezo [a,g]diphenylene-5,11-(6H,12H)dione in the amount of 88.5 g was also accomplished without difficulty.

This facility provided us with radiolabeled testostere-4-^{13}C-17β-heptanoate enriched with a stable C-13 isotope (99%) for metabolism studies.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis of specific steroidal and nonsteroidal compounds for evaluation for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the preparation of specific chemical compounds required by the CDB and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title	:	Analysis of Data from the Study of Correlation of Cervical Mucus Changes with Serum Hormone Levels
Contract No.	:	NO1-HD-8-2825
Contractor	:	Creighton University
Money Allocated	:	$22,564 (FY 78), $22,093 (FY 79)

Objectives: Under a previous contract (NO1-HD-6-2823) numerous data were acquired relating menstrual cycle phenomena to the fertile period and presumptive ovulation time. The objective of the present contract is to analyze the data and to discover whether objective practical determinants of the fertile period can be derived from the data.

Major Findings: Four points on the BBT curve have been correlated with the estimated time of ovulation (ETO), as determined by indirect hormonal parameters, in 74 menstrual cycles from 24 subjects. Only 10 of 66 hormonally normal cycles exhibited a BBT dip (D) and the ETO ranged from D-2 through D+3. In 63 of 66 hormonally normal cycles, a BBT nadir (N), first day of BBT rise (F) and BBT coverline endpoint (C) were identified. In these 63 cycles, the ETO ranged from N-5 through N+4, F-6 through F+3 and C-5 through C+4. Biphasic curves were observed in 72 of the cycles (97.3 percent) and monophasic curves in two cycles (2.7 percent). In at least five of 74 cycles (6.8 percent) the BBT curve gave incorrect information on the ovulatory status of the cycle.

Significance to Biomedical Research and Program of the Institute: Utilization of the mucus cycle as a method for fertility regulation is a subject of current investigations. Research relating the mucus cycle to hormonal status is directly related to investigations supported by the Contraceptive Development Branch.

Proposed Course: Initially it was felt that analyses would be completed during FY 1979. The new projection for completion is FY 1980.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Pharmacokinetics of Testosterone Enanthate in the Human Male
Contract Number : NO1-HD-8-2830
Contractor : Professional Staff Association of Los Angeles
Money Allocated : $66,875 (FY 78), $58,283 (FY 79)

Objectives: The proposed investigation will combine tracer kinetic studies of labelled testosterone enanthate (testosterone 7-H^3 enanthate) administered intramuscularly and intravenously on separate occasions into 7-10 normal male subjects with compartmental modeling to obtain estimates of the following pharmacokinetic parameters: plasma appearance rate; biological half-life; plasma (metabolic) clearance rate of testosterone enanthate, testosterone and estradiol; rate of conversion of testosterone enanthate to testosterone and estradiol; and the urinary excretion rate of testosterone enanthate and its metabolic products.

Major Findings: Significant progress has been made in the assessment of the pharmacokinetics of testosterone enanthate in the human male. 200 mg of testosterone enanthate (TE) has been injected intramuscularly in 7 subjects. Serum testosterone, estradiol, LH and FSH were measured before injection and at 6 hours, 1, 2, 3, 4, 7, 9, 11, 14, and 21 days after treatment. Basal serum T levels were 532 $\pm$ 58 ng/dl and rose rapidly to levels 226 $\pm$ 92% above baseline at 6 hours and peaked at 24 hours after injection (269 $\pm$ 64% above baseline; 1863 $\pm$ 231 ng/dl). Testosterone levels then declined to 33 $\pm$ 5% above baseline at 1 week and baseline -10 $\pm$ 7% at 9 days, remaining at this level until 3 weeks. Serum estradiol paralleled the testosterone levels, although the peak response lagged one day behind serum T. Mean baseline E^2 was 24.9 $\pm$ 3.1 pg/ml; peak E^2 was 44.9 $\pm$ 4.0 pg/ml, falling to baseline between days 7 and 9. Serum LH and FSH fell rapidly with the first significant decrement at day 1 (-31 $\pm$ 6% of baseline). The maximum decreases in LH and FSH were seen between days 4 and 9 (-61 $\pm$ 4% and -71 $\pm$ 5%). Three subjects have received ^3H TE combined with 200 mg of TE. Preliminary analysis from the tracer study indicates that: 1) The majority of testosterone appearing in the blood is the result of hydrolysis of TE in the muscle depot site. TE is also absorbed unchanged into the blood compartment. Testosterone appears in the plasma at 0.004% dose per liter within 30 minutes of the initial tracer intramuscular dose of testosterone enanthate. The peak in plasma testosterone (.026% dose per liter) occurs between 8 and 12 hours later. Testosterone enanthate and dihydrotestosterone follow a time course similar to testosterone.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of the new male methods of contraception.

Proposed Course: This is an eighteen month contract which should fulfill the stated objectives.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Development and Testing of Vaginal Contraceptives
Contract Number : NO1-HD-8-2856
Contractor : Cedars-Sinai Medical Center
Money Allocated : $124,338 (FY 78), $132,069 (FY 79), $60,741 (FY 80)

Objectives: The objective of the proposed study project is to determine the use-effectivensss and the acceptability of an intravaginal contraceptive, Encare Oval. The use-effectiveness and acceptability rates of the Encare Oval will be compared to those of a control group using the diaphragm with spermicidal gel or cream as a contraceptive method.

Major Findings: Patient enrollment began January 3, 1979, according to a randomized protocol. Randomization presented two problems, the first being that few patients would accept random assignment of a contraceptive method. The second, those accepting randomization appeared to be a very poorly motivated group of contraceptive users.

1. There appears to be large numbers of patients present at the clinics who have used the product, are using, or plan to use Encare Oval.

2. There is a very high incidence of a sensation of burning during intercourse when the Encare Oval is used (at least 30%). Presently there is no evidence of an unacceptably high pregnancy rate in our study patients.

3. Growing awareness of this side effect is preventing patients from participating in the study, and will probably contribute to a very high drop out rate of those participating.

4. Efforts must be directed at reaching a greater number of potential study participants and there should be a consideration of a means of collecting data from patients who have used or are using the product, but are not willing to commit themselves to formal participation in the study at this time.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of the new female methods of contraception.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Clinical Investigation of a Polyurethane Contraceptive Vaginal Sponge Containing a Spermicidal Agent
Contract Number : NO1-HD-8-2857
Contractor : University of Southern California School of Medicine
Money Allocated : $31,081 (FY 78)

Objectives: The purpose of this project is to evaluate the ability of a polyurethane contraceptive vaginal sponge containing nonyl-9 as a spermicidal agent to prevent the penetration of spermatozoa into and the migration of sperm through human cervical mucus. The effect of the sponge on the vaginal and cervical mucosa after seven days use will also be determined.

Major Findings: The study of the medicated polyurethane vaginal contraceptive sponge is divided into two parts: 1) Testing of tolerance to continuous wear for seven consecutive days, and 2) Evaluation of the ability of the sponge to prevent sperm penetration into and migration through the cervical mucus. In the initial phase of the irritation study, 10 subjects wore the one sponge during seven days that included the middle of the menstrual cycle. Three subjects completed the test without any major problems, four expelled the sponge after two to five days of use, and two women, after using the sponge for four days, developed a profuse, foamy drainage per vagina that caused a contact dermatitis on the vulva and inner aspects of the thighs. The protocol was subsequently changed so that the sponge was still used for 7 days, but not for longer than 48 hours. This regimen has avoided the problems incurred in the first phase of the study. The effect of the medicated sponge on sperm transport is being compared with non-medicated sponges and contraceptive foams. Using the fractional post coital test (PCT) done 1 to 2 hours after coitus, the medicated sponge had a variable effect on sperm transport. In some instances, the sponge was displaced no longer providing a mechanical barrier before the cervix. Instructions were modified to ensure that the sponge was thoroughly moistened before insertion so that foam was present on the surface, enhancing diffusion of the spermicide into the cervical mucus to provide a chemical barrier to sperm transport even if the sponge were displaced during intercourse. With this modification, the medicated sponge appears to be at least as effective as contraceptive foam in preventing sperm transport. After intercourse when fluid is expressed from the medicated sponge, all of the sperm present are non motile.

Significance to Biomedical Research and Program of the Institut : The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of the new female methods of contraception.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Development and Testing of Vaginal Contraceptives
Contract Number : Contract not awarded yet
Contractor : International Fertility Research Program
Money Allocated : Approx. $194,000 (FY 79)

Objectives: It is proposed to carry out a multi-clinic Phase III clinical trial of a newly developed non-prescription vaginal contraceptive method, the Collatex Sponge, in comparison with the diaphragm with spermicide. The incidence of adverse side effects and complaints associated with the use of the Collatex sponge compared with the diaphragm will be evaluated. Further, the rates of user compliance for the Collatex sponge will be determined. The sponge affords several significant advantages over the diaphragm. First, a single size can be used by all women; second, it is intended to be a non-prescription contraceptive; third, it is less messy than other contraceptives and can be inserted up to 24 hours in advance of coitus.

Major Findings: None. Contract not awarded yet.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of the new female methods of contraception.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Evaluation Branch
Center for Population Research

The Contraceptive Evaluation Branch is responsible for a comprehensive research contracts program on the evaluation of the safety of methods of contraception and fertility regulation which are in use or under development. Major program components deal with the determinations of the medical effects associated with the use of oral contraceptives, of intrauterine devices, of sterilization, and of possible effects of prior induced abortion on subsequent reproduction. Recognizing that an understanding of the pathogenetic mechanisms by which serious adverse medical effects of oral contraceptives arise, research into these aspects have received high priority and will continue to be emphasized in the time ahead.

Steroid Contraceptives

Research to determine whether steroid contraceptives increase the risk of breast cancer continues to receive high priority. A comprehensive, large-scale case control study of the relative risk of breast cancer among women on oral contraceptives is in progress under an interagency agreement with the Center for Disease Control. This project will take several years for completion.

A collaborative study of the relative risk of pituitary adenoma among women on contraceptive steroids is making good progress. Two case control studies of the relative risk of malignant melanoma in relation to oral contraceptive use are expected to complete data collection during the coming year.

A study of the effects of medroxyprogesterone acetate (MPA) in beagle dogs has provided a clue to the possible mechanisms involved in the development of mammary nodules. Marked elevation in growth hormone levels in beagles treated with MPA were correlated with the development of large mammary nodules. It is proposed to explore this observation further to determine whether growth hormone alone induces mammary nodules in beagles and whether this is the essential step in the sequence of events from MPA exposure to the development of mammary nodules.

A large case control study about the relative risk of myocardial infarction on women on oral contraceptives has confirmed the observations previously made in Great Britain. An increased relative risk was observed principally among women on oral contraceptives who had other important risk factors for cardiovascular disease, primarily cigarette smoking. Past oral contraceptive use did not appear to increase the risk of myocardial infarction.

A case control study is in progress, as recommended by the National Commission on Diabetes, to determine the relative risk of Diabetes Mellitus in relation to exposure to contraceptive steroids. Also, an RFP was issued to study the correlation between tryptophan and vitamin B_6 status and mood changes among women on oral contraceptive steroids, and to conduct a clinical trial provided this correlation has been confirmed. The study is about to be initiated with this year's funds.

The Walnut Creek Contraceptive Drug Study is phasing out and will terminate in January 1980. A comprehensive analysis of the data collected under this contract is in progress and will be available in monograph form as a final report from this study. Moreover, the Government has requested the data tapes from this contract for use as a resource in pursuing other questions relative to oral contraceptive safety that can be addressed by use of this data file.

Long-Term Effects of Sterilization

A finding of considerable significance was the observation of an increase in the extent and severity of atherosclerosis in the major arteries of rhesus monkeys vasectomized 9 to 14 years before sacrifice, as compared with age-matched controls. This finding provides increased importance to the ongoing cohort study to determine possible long-term effects of vasectomy in men. An RFP has been issued which proposes to focus on the possible increased risk of vascular lesions including myocardial infarction in relation to vasectomy.

Requests for reanastomosis by men are not at all uncommon because of changing circumstances. While some have claimed high rates of successful reanastomosis by microsurgical techniques, an objective evaluation is necessary. It is felt that if reanastomosis could assure restoration of fertility, it would undoubtedly enhance the acceptability of vasectomy by younger men. Also, the acceptability of female sterilization would be enhanced, providing reversibility can be assured, when required. A program is thus under consideration for next year to test the rate of success of current procedures used to reverse male and female sterilization and to study factors which may be associated with low success rates.

Studies will also be initiated to pursue possible long-term effects of female sterilization which have been suggested by several reports, of an increased rate of hysterectomy among women who had undergone female sterilization several years earlier.

Proceedings of a workshop on vasectomy entitled Vasectomy: Immunologic and Pathophysiologic Effects in Animals and Man, edited by Lepow and Crozier, have recently been published by Academic Press.

Hazards Associated with IUDs

A collaborative project to determine the relative risk of major complications associated with intrauterine devices has been completed and the analysis of the data is in progress. Comprehensive analyses of the relative risk of various complications including ectopic pregnancies, pelvic inflammatory disease, severe vaginal hemorrhage, fetal loss, abruptio placentae and placentia previa are expected during the coming year.

Effects of Induced Abortions on Subsequent Pregnancies

A report based upon data available from the Kaiser Hospitals in the Northern California region clearly revealed a small, but significant increase in risk in the incidence of second trimester abortions in women with one or more prior

induced abortions. This finding is also supported by preliminary data from two still ongoing contracts which are in various stages of data analysis.

Workshops

The Contraceptive Evaluation Branch sponsored a small workshop in February, 1979 attended by Dr. and Mrs. J. L. Beaumont, M.D., Scientific Director of the Research Unit on Atherosclerosis, Henri Mondor Hospital, Creteil, France to discuss his observations about immune complexes and other abnormal proteins which he has described in the serum of users of oral contraceptives. Beaumont has noted these complexes in a very high proportion of women who had developed atheriosclerosis while on oral contraceptives. Moreover, he observed the appearance of these complexes in the serum of women several months after initiation of oral contraceptive use with approximately 20% of these achieving rather high levels. His observation may represent a breakthrough of our understanding of the thrombotic complications of steroid contraceptives and may enable us to identify women at special risk. An RFP has been issued, first of all to attempt to independently confirm this observation, and when confirmed, to pursue the chemical and immunological nature of this immune complex.

In May, 1979, the CEB jointly with The Population Council in New York City sponsored a workshop about the effects of estrogens and progestins on various lipids and lipoprotein fractions. This workshop was stimulated by an observation derived from contract supported research indicating that certain progestins appeared to depress serum HDL levels. Since DHL is thought to be protective against myocardial infarction, this effect of progestin is of critical importance. Moreover, investigators supported by The Population Council who are working on the development of a vaginal ring which contains progestin to be released locally for contraception action, observed a similar decrease in HDL levels with this mode of administration of progestin. The conference provided an excellent summary of the state of our current knowledge and identified several new avenues for research of relevance to this program.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title : Contraceptive Steroids in Beagles
Contract Number : NO1-HD-2-2725
Contractor : Cornell University
Money Allocated : $115,000 (FY 78 funds)

Objectives: The purposes of this project have been to develop assays for measuring circulating levels of reproductive hormones in the beagle, to describe hormonal control of the normal reproductive cycle in this species, and to study the effects of contraceptive steroids on endocrine function, particularly in relation to the development of steroid-induced mammary nodules.

Major Findings: The normal reproductive endocrinology of the beagle has been described in detail and it is clear that the basic hormonal mechanisms controlling the estrous cycle, pregnancy and parturition are very similar to those of other species. The only difference is that the beagle apparently lacks an acute luteolytic mechanism and so has a prolonged period of luteal regression resulting in estrous cycles of 6-7 months.

Studies of the effects of medroxyprogesterone acetate (MPA) in the beagle have provided a new clue to possible mechanisms involved in the development of progestin-induced mammary nodules. Data recently submitted for publication demonstrate a marked elevation in growth hormone levels in beagles treated with MPA at high dosage for 17 months. The elevated growth hormone levels (12.8 ± 3.0 vs 0.5 ± 0.1 ng/ml in controls) were correlated with development of large mammary nodules and the appearance of acromegaly. Progesterone treatment did not have similar effects, and prolactin levels were unchanged by treatment with either MPA or progesterone.

Significance to Biomedical Research and the Program of the Institute: The Food and Drug Administration requires long-term toxicity testing in beagles and monkeys for steroids proposed for contraceptive use. A number of progestins have been removed from the market because of the development of mammary nodules in beagles but the nature of these tumors and their relevance to human cancer have been controversial. The endocrine data from this contract demonstrate that beagle reproductive endocrinology is not sufficiently different from that of other species to invalidate the beagle as an animal model in steroid toxicity studies. The finding of elevated growth hormone levels in beagles treated with MPA and developing large mammary nodules is a significant clue to understanding the mechanisms which may be involved in induction of these lesions. It may be that the beagle lesions, whether spontaneous or the result of progestin treatment, are induced by growth hormone. If so, and if these progestins do not cause growth hormone elevation in women, the relevance of the beagle mammary nodules to possible human cancer would be more doubtful.

Proposed Course: Work under this contract has been completed. Studies to confirm and extend the recent finding of elevated growth hormone levels in beagles treated with MPA will be pursued under new contracts to be solicited next year.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title	:	Central Repository for Tissue Evaluation of Contraceptive Steroids
Contract Number	:	NO1-HD-2-2729
Contractor	:	Universities Associated for Research and Education in Pathology
Money Allocated	:	$117,733

Objectives: The purpose of this project is to evaluate the pathologic effects of contraceptive steroids in long-term toxicity studies, primarily in beagles and monkeys. Tissue specimens are obtained from studies conducted by pharmaceutical companies and others, and subjected to detailed pathologic evaluation at the Armed Forces Institute of Pathology.

Major Findings: Studies have been completed on all canine specimens from the FDA-sponsored study of three investigational progestins and mestranol. The malignant tumors occurring with these steroids are no different morphologically from those reported in surveys of spontaneous canine tumors, but they occurred at younger ages and anaplastic carcinomas were more frequent. Comparative pathologic studies of spontaneous canine mammary lesions have also been completed, including evaluation of early dysplastic lesions detected in whole mount preparations. These lesions were found in the majority of bitches aged 3-4 years, but not in younger animals. Myoepithelial cell hyperplasia, a frequent component in the small palpable nodules in steroid-treated beagles, is rare in these spontaneous mammary lesions.

Significance to Biomedical Research and the Program of the Institute: The Food and Drug Administration requires long-term toxicity testing in beagles and monkeys for steroids proposed for contraceptive use. A number of progestins have been removed from the market because of the development of mammary nodules in beagles, but the nature of these tumors and their relevance to human cancer have been controversial. This contract provides expert, unbiased evaluation of tissues from canine and monkey toxicity studies, and comparison with human pathology, as a guide in interpreting data from these and other studies.

Proposed Course: During the next year, tissues will be evaluated from canine studies of medroxyprogesterone acetate and progesterone and monkey studies of investigational steroids.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Secondary Amenorrhea, Pituitary Adenoma and Oral Contraceptives

Contract Number	Contractor	Money Allocated
NO1-HD-7-2834	Johns Hopkins University	$306,832
NO1-HD-9-2810	University of Southern California	133,575
NO1-HD-9-2811	Johns Hopkins University	147,509
NO1-HD-9-2812	University of California, San Francisco	174,679

Objectives: These contracts support a collaborative case-control study to determine relationships between secondary amenorrhea with and without pituitary adenoma, and previous use of oral contraceptives. Patients meeting specified criteria for secondary amenorrhea and/or galactorrhea, prolactin-secreting pituitary adenoma, or elevated prolactin levels, are interviewed to determine their contraceptive history. Data for comparison are obtained from matched normal controls. The data will be analyzed to determine whether oral contraceptive use previous to diagnosis was more common or of longer duration in patients in one or more of the disease categories than in the controls.

Major Findings: The detailed protocol, questionnaire and manual of procedures for this study have been developed and data collection has begun. Abstracting of medical records is essentially complete at one of the centers and well under way elsewhere, and interviewing has been initiated.

Significance to Biomedical Research and the Program of the Institute: Pituitary adenomas are being diagnosed in increasing numbers of women with secondary amenorrhea. This may result from improved radiographic and radioimmunoassay techniques which can now identify very small tumors, or it may be that these tumors are increasing in frequency. Many of these tumors have been reported in women who have discontinued use of oral contraceptives. This study should determine whether there is a correlation between the occurrence of pituitary adenomas and previous pill use, and should distinguish a possible causal relationship from the reappearance of a tumor present before pill use but masked by the steroid medication.

Proposed Course: Data collection in this study is expected to require another year, and analysis of the data will require a further six months.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Evaluation Branch
Contract and Collaborative Research

Interagency Agreement Title:	Cancer and Steroid Hormones
Interagency Agreement Number:	YO1-HD-8-1037-00
Agency:	Center for Disease Control, Atlanta, Georgia
Money Allocated:	$155,134

Objectives: To evaluate the relationship between oral contraceptive use and the occurrence of breast, endometrial, and ovarian cancer by means of a population-based case-control study of incident cases of breast, endometrial, and ovarian cancer.

Major Findings: A study protocol, training manual (for interviewers) and questionnaire have been developed. A pilot study is under way to field test the study procedures and data instruments and to assess the cost of full-scale deployment of the study.

Significance to Biomedical Research and Program of the Institute: This study is designed to rigorously address the question of whether oral contraceptive use affects the occurrence of breast, endometrial, and ovarian cancer.

Proposed Course: Upon completion of the pilot study, the feasibility and desirability of proceeding with the full-scale study will be assessed. It is anticipated that the full-scale study will be undertaken, and will begin in the Spring of 1980.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title : Case-Control Study of the Relative Risk of Malignant Melanoma of the Skin Among Women on Contraceptive Steroids
Contract Number: NO1-HD-8-2803
Contractor : University of California, Los Angeles, California
Money Allocated: $35,948 from FY 1978 Funds
$135,322 from FY 1979 Funds
$103,172 from FY 1980 Funds

Objectives: These are to estimate the relative risk of malignant melanoma in women exposed to steroid contraceptives taking confounding variables of sunlight exposure and sun sensitivity into account. One-hundred-fifty incidence cases are to be ascertained from the two population-based tumor registries in Los Angeles and the five county San Francisco Bay area. Incidence cases are preferred to prevalence cases since the effect of steroid contraceptives on survival time is not known and to avoid errors in recall and other biases in the interpretation and generalizability of results. For each case two controls are to be selected, one of which is to be a longstanding friend, the second a neighbor. Both are to be matched for age and skin color. They will serve as partial controls for life-style, sun exposure and sun sensitivity, judged from complexion and skin color. Variables not matched in the design are to be analysed as covariates. Both matched pair and covariate analysis are to be used.

Major Finds: None have yet been made. Progress consists of the following operational accomplishments: (1) The necessary questionnaire was developed. (2) OMB clearance of the questionnaire was obtained. (3) A Manual of Operations was developed. (4) A coding form was designed and coding instructions developed and tested. (5) Interviewing has been initiated.

Significance to Biomedical Research and Program of the Institute: A major concern with safety of oral contraceptives is their potential, casual association with malignant diseases. Malignant Melanoma of the skin is one of several clinical entities for which such an association has been suggested but not established.

Proposed Course: Due to unavoidable delays in establishing the operational prerequisites for interviewing, the contract period for data collection and analysis had to be extended for 9 months, until December 12, 1980.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title : Case-Control Study of the Relative Risk of Malignant Melanoma of the Skin Among Women on Contraceptive Steroids
Contract Number: NO1-HD-8-2804
Contractor : University of Sydney, Sdyney, Australia
Money Allocated: $72,030 from FY 1978 and FY 1979 Funds

Objectives: These are to characterize the association between use of oral contraceptives and occurrence of malignant melanoma, the determination of relative risk, causative and confounding factors. The most recent 300 prevalent cases in women aged 15-49 years, diagnosed since 1970 are to be ascertained from the files of the Sydney Hospital, Melanoma Unite. Further, 100 incident cases are to be ascertained as they are diagnosed in Sydney Hospital during the first 18 months of this contract. For each case selected, one neighborhood and one hospital control, matched by year of birth, is to be ascertained.

Cases and controls are to be interviewed using a questionnaire to obtain contraceptive reproductive history and sunlight exposure. The presence of confounding factors will be sought by stratification of data according to age, parity, marital status, sunlight exposure, skin color or sun sensitivity. It is projected to be able to detect a relative risk of 1.8 with 90% certainty at a 1% significance level.

Major Findings: The project was initiated on May 16, 1978. By the end of June 1979, 41 new melanoma cases, 175 old melanoma cases and 331 controls had been interviewed. Preliminary analysis suggests that if oral contraceptives increase the risk of melanoma, the relative risk is small. Thus 1200 interviews are needed to define the risk with any precision.

Significance to Biomedical Research and Program of the Institute: This is marked, since a major concern with safety of oral contraceptives is their potential, casual association with malignant diseases.

Proposed Course: The interview period needed and was extended by 6 months, i.e. from December 1979 to June 1980. This is to be followed by the analytic phase of the project. The final report is then to be prepared by March 1981, rather than by July 1980, as initially intended.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Decreased Risk of Breast Disease Among Oral Contraceptive Users: Histopathologic Evaluation of Epithelial Atypia
Contract Number: NO1-HD-9-2813
Contractor: Stanford University, Stanford, California
Money Allocated: $75,000

Objectives: To evaluate whether the reduced risk of benign breast disease which is associated with oral contraceptive use of about two to four years duration or longer varies for different histopathologic forms of benign breast disease.

Major Findings: A suitable epidemiologic and histopathologic data base for this new project has already been established under previous contracts with NICHD and NCI. Analysis of that data base comprises the work to be conducted under this new contract.

Significance to Biomedical Research and Program of the Institute: One published study indicates that oral contraceptive use of about two to four years or longer decreases the risk of those histopathologic forms of fibrocystic breast disease (the most common form of benign breast disease) which are not, or are only weakly, associated with an increased risk for subsequent breast cancer. That study suggests that oral contraceptive use does not decrease, and may increase risk for those histopathologic forms of fibrocystic breast disease which are more strongly associated with increased risk for subsequent breast cancer. This new contract will provide additional data on this important issue.

Proposed Course: This project has just begun. Completion in one year is anticipated.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title : Effects of Contraceptive Steroids on Blood Pressure
Contract Number: NO1-HD-75-2830
Contractor : Evans County Health Department, Claxton, Georgia
Money Allocated: $162,038 from FY 1978 funds
$114,587 from FY 1979 funds

Objectives: This is a prospective cohort study to determine change in blood pressure before and subsequent to initiation of contraceptive steroids, in a biracial population residing in the southeastern hypertension stroke belt in southeastern Georgia. Undefined geographic peculiarities in this region determine the highest mortality from hypertensive stroke for both whites and blacks in the United States. This study will answer the question whether the geographic determinants also effect an increase of hypertension associated with the use of oral contraceptives. Effects of predisposing, hereditary factors on contraceptive induced blood pressure elevation are to be investigated. Family histories are to be analysed and siblings of cohort members are to be examined.

Major Findings: The protocol specified enrollment of 1,000 pill choosers and 1,200 IUD choosers as controls, matched for age and race, subsequent examination at three month intervals for a study period of 3 years. So far only about half of these operational tasks have been accomplished. The expected hypertensive effect of oral contraceptives has not, as yet, been demonstrated. On the contrary the follow-up examinations in OCA-users, after 3 months of contraceptive use were associated with a decrease of blood pressure, as was found among the controls.

Significance to Biomedical Research and Program of the Institute: In conjunction with the findings from Atlanta (-HD-5-2831) and Boston (-HD-5-2832), the forthcoming findings of this Evans County project might shed unexpected light on what once was believed the most frequent major adverse health effect of OCAs, i.e. hypertension. This might bring about a re-examination of current concepts regarding the operation and arithmetic of risk factors for adverse OCA effects and hypertension. The operational task of this project, i.e. recruitment and examination of potential OCA-user, before using these drugs and subsequent prospective surveillance during OCA-use has never before been attempted. It proved to be a formidable task, which accounts for the length of the project period. This might be significant for anticipation of similar difficulties in other projects, currently under review, which attempt a similar task.

Proposed Course: The project was initiated in June 1975 and is expected to be completed in March 1980; according to the presently operative protocol.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title : Effect of Contraceptive Steroids on Blood Pressure
Contract Number: NO1-HD-5-2831
Contractor : Emory University
Money Allocated: $275,100 from FY 1977 funds

Objectives: 1) Estimation of prevalence, incidence and magnitude of blood pressure elevation in a cohort of black contraceptive users in Atlanta, Georgia. 2) Assessment of variables, determining and confounding this association.

Major Findings: No significant ($p<0.05$) association between oral contraceptive use and blood pressure of 20,639 southeastern women, examined between September 1975 and August 1978, was found. This conclusion is based on four categories of data: (1) The prevalence and incidence of hypertension in oral contraceptive users were not higher than in non-users. Over 1,000 women with elevated blood pressure were identified equally frequent in users than in non-users. (2) The family history of hypertension does not differ between hypertensive contraceptive users and women with essential hypertension. (3) The clinical and laboratory features and medical histories of hypertension in oral contraceptive users do not differ from essential hypertension without contraceptive therapy. (4) The spontaneous and therapeutic reversibility of hypertension in contraceptive users do not differ from essential hypertension in the absence of contraceptive therapy.

Significance to Biomedical Research and Program of the Institute: These findings are contrary to all previous reports, which gave rise to John H. Laragh's published opinion: "By conservative standards about 5% of women using oral contraceptives develop overt hypertension." This number of contraceptive induced hypertension, in about half a million women in the United States, would be larger than the combined frequencies of all other diseases attributed to adverse effects of OCAs. Since it is unlikely that all previous estimates were wrong, the above findings from Emory demand an explanation: Either the population of OCA-users has changed, or the composition of the OCA's, they are using, has changed!

Proposed Course: The project is completed.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title : Effects of Contraceptive Steroids on Blood Pressure
Contract Number: NO1-HD-75-2832
Contractor : Harvard Medical Schook, Boston, Massachusetts
Money Allocated: $138,825 from FY 1977 funds

Objectives: In 1973 a total community survey on all 5,000 white females, ages 16-49 years, residing in East Boston was conducted by the National Heart and Lung Institute (HDFP). In this survey blood pressure, contraceptive use and demographic characteristics were ascertained. This provides the basis for the objectives of this project: 1) Determine incidence of blood pressure changes associated with continued, discontinued or initiated contraceptive therapy since 1973. This is to be done by a second total community survey in 1976-1977. Blood pressure changes since 1973 are to be related to a) continuous OCA-use, b) continuous non-OCA-use, c) initiation of OCA-use, d) discontinuation of OCA-use. 2) Perform a third follow-up survey in 1978 on all women previously identified as a) contraceptive users, b) hypertensive and c) a random sample of 1,000 non-hypertensive non-users of oral contraceptives. 3) Determine characteristics associated with blood pressure changes and contraceptive use.

Major Findings: According to the variable of contraceptive use the cohort comprises 4 subcohorts: (a) 1973 No/1976 No = continuous OCA-non-user, (b) 1973 Yes/1976 Yes = continuous OCA-users, (c) 1973 Yes/1976 No = discontinuing OCA-users, (d) 1973 No/1976 Yes = OCA-use initiators. The preliminary, partial analysis restricted itself to relating the two variables of changing contraceptive use to changes in blood pressure. This revealed no marked hypertensive drug effect, whether such an effect was minimal or absent could not yet be determined. This uncertainty results from a third variable: continuous non-OCA-users were 10-15 years older and also heavier than the cohort of new OCA-users. The other two subcohorts were intermediate. This age difference between the subcohorts, defined by OCA-use status might account for as much or more than 5 mm Hg difference in blood pressure and precludes simple interpretation of the variable, blood pressure in terms of the variable contraceptive use. Furthermore OCA-use may be associated with blood pressure elevation in heavier females, more than 25-30 years old, but not in adolescent teen-agers, less than 20 years old.

The still forthcoming final analysis suggests a slight or minimal hypertensive effect of OCAs; possibly contrary to the Emory findings in Atlanta, Georgia, on blacks, where not even a minimal effect was found. An additional important finding from Boston seems more firmly established: OCA-use was associated with an increased risk of "persistent" bacteriuria. All or most other established effects of these drugs are transient; only determined by current use, disappearing after discontinuation.

Significance to Biomedical Research and Program of the Institute: The "Boston findings" on whites might provide an essential clue for explaining the "Atlanta findings" on blacks.

Proposed Course: The operations of the project have been completed on March 31, 1979. We are waiting for the final analysis and the final report.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Evaluation Branch
Contracts and Collaborative Research

Contract Title: Death from Myocardial Infarction and the Role of Oral Contraceptives
Contract Number: NO1-HD-6-2808
Contractor: Biostatistics Center, George Washington University
Money Allocated: 0

Objectives: To evaluate the relationship between oral contraceptive (OC) use and the occurrence of fatal myocardial infarction (MI) among women of reproductive age during the interval January 1, 1974 to June 30, 1975. This is the most accessible period preceeding publicity of two British studies which reported an increased risk of MI among OC users.

Major Findings: Data concerning 163 women (cases) who died of acute MI or "sudden death" (presumed to be mostly acute MI) during the study interval in the five largest Standardized Metropolitan Statistical Areas in the United States were compared to similar data for 163 women who died from other causes and 163 women who were hospitalized and survived, (i.e., there was one deceased and one living control per case). Cases and controls were matched for age, city, and year of death (or hospitalization).

Among nonwhite women, no association between OC use and the risk of fatal MI was found. For white women, the results were consistent with an increased risk of fatal MI among OC users (as compared to nonusers) of about two fold. There was no evidence of a synergistic effect of OC use and smoking on the occurrence of fatal MI.

Significance to Biomedical Research and Program of the Institute: The results of this study provide support for the belief that OC use increases the risk of fatal MI among women of reproductive age, but provides no support for the widely held belief that the synergistic effect of OC use and smoking on the occurrence of non-fatal MI also exists for fatal MI.

Proposed Course: The contract has terminated.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Case-Control Study of Myocardial Infarction in Young Women with Special reference to the Role of Oral Contraceptives
Contract Number: NO1-HD-6-2849
Contractor: Boston University, Boston, Massachusetts
Money Allocated: $394,328

Objectives: To evaluate the relationship between oral contraceptive (OC) use and the occurrence of non-fatal myocardial infarction (MI) among premenopausal women. Special emphasis is being placed on evaluation of possible interactions between OC use and other MI risk factors such as cigarette smoking, hypertension, blood lipids, etc.

Major Findings: Two hundred thirty-four premenopausal women with first MI have been compared to 1742 hospitalized controls. (Lancet 1:743-747, 1979). The overall risk of MI for current OC users (within the month preceeding hospitalization), relative to the risk for non-users, was estimated to be 4 (95% of CI: 2.5-6.3). For women who smoked heavily but were not current OC users, relative to women who did not smoke and were also not current OC users, the risk estimate was 7 (95% CI:5-12). For women who both smoked heavily and were current OC users, relative to women who did not smoke and were not current OC users, the risk estimate was 39 (95% CI:22-70). Past OC use (prior to the month preceeding hospitalization) did not appear to increase MI risk.

Significance to Biomedical Research and Program of the Institute: The relationship between OC use and the occurrence of MI is on issue of substantial public health importance. This project is providing data which are needed to clarify this relationship.

Proposed Course: Collections of data from new cases and controls ceased in June, 1979. All available cases and an appropriate sample of controls will be reinterviewed at home following hospital discharge. A blood specimen will be obtained for cholesterol, triglyceride, high density lipoprotein, and ABO blood type determinations. A type-A, type-B personality profile will be obtained. These data will be incorporated into the existing data file (demographic characteristics, medical, surgical, reproductive, and birth control history, smoking, alcohol use, etc.), and the relationship between OC use and the occurrence of MI will be reanalyzed to evaluate the relationships between all of the risk factors for which data have been obtained.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: The Effect of Oral Contraceptives on Idiopathic Venous Thrombosis: A Study of Antithrombin III and Fibrinolysis
Contract Number: NO1-HD-7-2804
Contractor: Duke University Medical Center
Money Allocated: 0

Objectives: To test the hypothesis that women who develop venous thromboembolic diseases during oral contraceptive use have intrinsically lower fibrinolytic response following venous occlusion than do women who do not develop these diseases during oral contraceptive use.

Major Findings: A reliability study was conducted to estimate the biologic and measurement variation of a 5 minute venous occlusion procedure for measuring activators of fibrinolysis among a sample of 20 healthy women ages 21-35 years. The rank orderings of subjects' fibrinolytic response (FR) to venous occlusion were similar at two visits on different days; the measurement showed wide variation. FR was observed to vary in relation to habitual physical exercise, smoking, and body weight. Minimal diurnal variation was observed.

A case-control study was conducted comparing 17 cases of idiopathic thromboembolism and 29 age and race matched controls. 76% of cases and 83% of controls had used oral contraceptives (OC) in the past and all had stopped using OC at least 12 months prior to study. Among subjects who had used OC, the odds ratio of low FR was 7.2. These data suggest that the relative risk exceeds one with 95% confidence. The mean difference in FR between all cases and controls was -30 mm^2 (with FR truncated at 250 mm^2). The mean rank ordering of cases was 7 ranks lower than controls. Pooling all subjects, the odds ratio of low FR was 4.0. The lower limit of the one-sided 95% confidence interval was one.

Significance to Biomedical Research and Program of the Institute: The results of this project indicate that the risk of thromboembolic disease during OC use may be increased among women with relatively low fibrinolytic response.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title : Effects of Oral Contraceptives on Folic Acid Metabolism
Contract Number: NO1-HD-74-2862
Contractor : University of Alabama School of Medicine
Money Allocated: $98,797 - from FY 1978 funds
$79,115 - from FY 1979 funds

Objectives: These objectives are based upon striking findings of unknown medical significance by Whitehead, Reyner and Lindenbaum. Cervical smears in 19% of contraceptive steroid users revealed "undefined" morphological abnormalities. They were similar to those (a) accompanying erythropoetic megaloblastosis found in Vitamin B_{12} and folate deficiency and (b) of the premalignant lesion: cervical dysplasia. In spite of absence of systemic folate deficiency, administration of monoglutamic folic acid (10 mg daily for 3 weeks) reversed or cured all megalocytotic abnormalities. It has never been investigated whether these morphologic abnormalities in the absence of folate therapy may actually progress towards cervical dysplasia and whether they reflect local functional folate deficiency in the regenerating cervical epithelium. The objectives of this project were to: (a) determine whether oral contraceptives have a selective, localized effect on folate metabolism in the endocervix leading to a functional folate deficiency in this target tissue (animal experiments), (b) assess the efficacy of folate therapy for reversing cervical dysplasia in users of oral contraceptives (clinical trial).

Major Findings: The results of the animal experiments (a) have been described in last year's progress report. They provided the scientific basis and rationale for the still ongoing clinical trial (b) under the title: Folate-Induced Regression of Cervical Dysplasia in Users of Oral Contraceptives, the following findings were published: 26 young women having OCA-associated cervical dysplasia of mild or moderate degree were randomly assigned to daily doses of either folic acid 10 mg or a placebo (ascorbic acid, 10 mg). All were followed by colposcopy and cervical cytology. Final vs. initial cytology scores were similar in the placebo group, 1.33 and 1.18 respectively; but lower in the folate group, 0.45 and 1.13. The ratio of progression/regression was 4/2 with placebo vs. 1/7 with folate. Conclusion: Folate supplementation may favorably influence the course of mild or moderate cervical dysplasia in OCA-users.

Significance to Biomedical Research and Program of the Institute: The possibility of a medical therapy of a premalignant lesion is suggested. Therapy is based on a nutritional intervention in its pathogenesis; that is, correction of abnormal nucleic acid synthesis resulting from folate dependent nucleotide synthesis.

Proposed Course: In order to establish statistical significance of the reported findings a sample size of at least 80 subjects is necessary. Since patients on oral contraceptives with mild or moderate cervical dysplasia did not and may not turn up in the expected number, the contract period may have to be extended, beyond January 29, 1980; its current expiration date.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title : Relative Risk of Diabetes in Users of Steroid Contraceptives
Contract Number : NO1-HD-8-2818
Contractor : Emory University
Money Allocated : $146,000

Objectives: The purpose of this study is to determine whether steroid contraceptive use, which is known to result in changes in glucose tolerance, is associated with an increased risk of overt clinical diabetes mellitus. The contractor will conduct a case-control study comparing contraceptive histories in 150 newly diagnosed diabetic women of reproductive age with histories in 450 control women matched for age, race and socioeconomic status.

Major Findings: Data collection in this study is well under way and interviews have been completed on 115 cases and 18 controls. (The controls lag behind the cases because of the need to identify matching characteristics in the cases before selecting controls.) A beginning has been made in planning for analysis of data from the study.

Significance to Biomedical Research and the Program of the Institute: Oral contraceptives are now used by millions of American women. Effects of the contraceptive steroids on glucose tolerance have been reported since the early 1960s, and it has been postulated that the steroid-induced changes in carbohydrate metabolism may, with continued exposure, lead to frank clinical diabetes. No evidence on this point has been obtained from general prospective studies of OC users and no retrospective studies of the risk of diabetes have been reported. This contract is expected to answer the question of the clinical significance of changes in glucose tolerance induced by oral contraceptive use and determine whether long-term users are at increased risk of developing diabetes.

Proposed Course; Data collection for this study is expected to be completed by January 1981 and data analysis will require a further six months.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title : Contraceptive Drug Study
Contract Number: NO1-HD-3-2710
Contractor : Kaiser Foundation Research Institute, Walnut Creek, California
Money Allocated: $332,705 (13½ months)

Objectives: This prospective longitudinal study of 18,000 women who are part of a prepaid medical plan in Walnut Creek, California is concerned with the identification of hazards associated with the use of contraceptive steroids.

Major Findings: This project has produced numerous findings, which are relevant to the determination of the safety of contraceptive steroids. These include an increased relative risk of thromboembolism, stroke and subarachnoid hemorrhage and a possible slight increase in carcinoma in situ of the cervix. Other findings of interest include an effect of oral contraceptives on certain lipoprotein fractions, glucose tolerance, and various coagulation factors.

Significance to Biomedical Research and the Program of the Institute: The determination of the adverse long-term effects associated with the use of oral contraceptives is critical to the program of the Center for Population Research and the NICHD. This is one of the major efforts related to this goal.

Proposed Course: This study will be phased out by January 15, 1980. The contractor is preparing a comprehensive analysis of the data collected under this contract for publication in monograph form. This report will summarize the information relative to the risk of various clinical diseases associated with the use of oral contraceptives including duration of use. The study is being phased out in view of the fact that the cohort under investigation has aged to the point that a relatively small proportion are still pill users. For this reason it will not be able to contribute very much more on the question of risk associated with current pill use.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Prospective Study of the Effects of Contraceptive Agents, Related Methods and Procedures on the Frequency of Birth Defects
Contract Number: NO1-HD-4-2861
Contractor: Kaiser Foundation Research Institute, Walnut Creek, California
Money Allocated: $39,300

Objectives: This project is concerned with the determination of the risk to the fetus of the use of contraceptive agents at or around the time of conception or early in pregnancy. Among the possible effects to be evaluated are birth defects, perinatal mortality, and low birth weight.

Major Findings: Analysis in progress at this time reveal a slight increase in the risk of second trimester spontaneous abortions among women with a history of prior induced abortions as compared to women without such a history. There appears to be a slight increase in risk of chromosome elaborations among breakthrough pregnancies in women on oral contraceptives at the time of conception. Data regarding effects of inadvertent hormonal exposure at and around the time of conception or early in pregnancy on the risk of congenital malformations are currently being analyzed.

Significance to Biomedical Research and to the Program of the Institute: Inadvertent exposure to contraceptive steroid hormones at or around the time of conception or early pregnancy is quite common. It is estimated to occur as often as in 1 to 2% of all pregnancies. For this reason, were it associated with a marked increase in the risk to the fetus, it could become a major public health issue. Therefore, the determination of the size of the risk, if any, of such exposure to the fetus is critical. The Center for Population Research as part of the NICHD's program, has a mandate to research adverse effects of contraceptive methods in use.

Proposed Course: This project is complete except for the analysis of the data. These analyses are in progress and are expected to be completed during the summer of 1979 at which time the contract will terminate.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Evaluation Branch
Contract and Collaborative Research Program

Contract Title: Maternal Factors in Birth Defects
Contract Number: NO1-HD-6-2800
Contractor: University of Maryland School of Medicine, Baltimore, Maryland
Money Allocated: 0

Objectives: To evaluate the relationship between oral contraceptive use at or around the time of conception and the occurrence of conotruncal heart malformations.

Major Findings: Exogenous female sex hormone exposure was studied in the mothers of 110 infants with conotruncal cardiac malformations. For each case, three controls were chosen from the birth population: Control I matched on 8 characteristics related to the likelihood of hormone-taking (race, maternal age, parity, fetal losses, gestational age, delivery mode, time of prenatal registration, private/service); Control II matched on these plus infant's sex and birthweight; Control III was chosen at random. Personal interviews covered general and pregnancy health, family history, personal and environmental exposures. Identification of hormones taken was aided by a display of pills and packages. Hormones taken in early pregnancy were: oral contraceptives in 5.5% of cases and in 2.5%, 5.4%, 5.0% of control groups respectively; pregnancy test in 3.6% and 5.1%, 7.6%, 3.4%, pregnancy maintenance in 4.5% and 2.5%, 3.3%, 7.7%. Multilogistic regression analysis controlling for matching variables and scores for reproductive, malformation and exposure risks revealed no association of prenatal hormone exposure with conotruncal heart disease.

Significance to Biomedical Research and Program of the Institute: Some published data suggest that exposure to oral contraceptives at or around the time of conception may increase the risk of heart malformations. This project provides evidence against an increased risk for conotruncal heart malformations.

Proposed Course: A detailed final report from this project is in preparation, and is scheduled for submission to the Institute June 30, 1979. The project will then terminate.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title : Long Term Effects on Mothers and Offspring of Diethylstilbestrol Treatment During Pregnancy
Contract Number: NO1-HD-8-2829
Contractor : University of Chicago
Money Allocated: $280,765 (FY 79)

Objectives: This project is to determine the effects of prenatal exposure to diethylstilbestrol (DES) on the health of male and female offspring especially on reproduction and the risk of cancer and also to determine whether there is a risk of cancer of the breast and other organs associated with the use of DES by the mothers.

Major Findings: Male offspring exposed in utero to DES revealed a greater proportion with minor abnormalities of the external genitalia including epidymidal cysts and also low sperm counts and decreased sperm mobility. Among female offspring no case of clear-cell adenocarcinoma of the vagina or cervix has been identified. A high proportion reveal vaginal and cervical adenosis and other related findings typical for DES in utero exposure. Among the mothers exposed to DES while pregnant a greater risk of cancer of the breast and of the ovary and cervix was reported after the first review of the cohort which was of borderline significance.

Significance to Biomedical Research and the Program of the Institute: This is a unique study based upon a controlled clinical trial to evaluate the effectiveness of DES in improving pregnancy outcome which was conducted at the Lying-In Hospital in Chicago during the early 1950s. The determination of adverse effects of prenatal hormonal exposure on the reproductive system of female and male offspring is of critical importance to the program of this Institute.

Proposed Course: Reevalutation of the female and male offspring is concerned with the determination as to the specific effect of DES exposure on the risk of infertility in the male as well as infertility or subfertility in the female. In addition, other adverse health effects including cancer of the uragenital tract are evaluated. Further followup of the mothers is aimed at resolving the question as to whether there is indeed an increase risk of cancer of the breast and reproductive organs associated with DES exposure in this cohort.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title : A Comparative Study of Progestin Metabolism and Mechanism of Action
Contract Number : NO1-HD-2-2730
Contractor : Pennsylvania State University
Money Allocated : $30,463

Objectives: This contract has supported comparative studies of the metabolism and biological effects of a series of progestational agents in monkeys, dogs, rats, mice, and human subjects. The investigations included determination of metabolic clearance rates, hepatic and extrahepatic metabolism, and the effects of the progestins on a wide range of biochemical parameters in various target tissues.

Major Findings: During the past year, studies have been completed describing nuclear responses to stimulation of androgen and progestin receptors. The data indicate that a major mechanism by which medroxyprogesterone acetate (MPA) expresses its biological effect is through augmentation of the transcriptional process, including increases in the number and/or activity of RNA polymerase molecules as well as induction of additional sites on chromatin for the initiation of RNA synthesis. In contrast, cyproterone acetate, an antiandrogenic progestin, had no effect on RNA polymerases and apparently increased transcription only via alterations in chromatin template activity. The responses to both steroids are absent in Tfm/Y mice, indicating that their expression is dependent on an effective androgen receptor.

Significance to Biomedical Research and the Program of the Institute: This wide ranging study has provided fundamental quantitative information on the effects of progestins in a variety of systems and several species. The information should contribute to a better understanding of the effects of these contraceptive steroids in women and to the development of methods for predicting the effects of specific compounds in animal tests.

Proposed Course: Work under this contract will be completed this year.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title : Effects of Vasectomy on Testis and Epididymis
Contract Number : NO1-HD-2-2770
Contractor : University of Virginia School of Medicine
Money Allocated : $73,333

Objectives: This contract supports animal studies of the effects of vasectomy on intraluminal pressure and electrolyte concentrations in the testis and epididymis and the permeability of the blood-testis barrier and epididymal wall. Micropuncture techniques are used to obtain fluid samples and pressure measurements. Light and electron microscopic studies are correlated with the studies of luminal contents to evaluate absorptive and secretory activity.

Major Findings: The effects of vasectomy on the blood-testis and blood-epididymal barriers to water, inulin, and urea have been examined in hamsters. Subtle changes in the function of these barriers were demonstrated at 4 months after vasectomy (increased entry of water and urea into the seminiferous tubules and of water and inulin into the epididymis, and decreased entry of urea into the epididymis). Studies are continuing on the localization of androgen binding protein in luminal fluids from the seminiferous tubules and epididymis in normal rats, and studies are nearing completion on electrolyte concentrations in reproductive tract fluids from normal and vasectomized hamsters and normal and hormone-treated rats.

Significance to Biomedical Research and the Program of the Institute: Data from this project are providing new information on the physiology of the male reproductive tract and alterations resulting from vasectomy. Physiological modifications not repairing themselves after vas reanastomosis may be a significant factor in persistent infertility after vasectomy reversal.

Proposed Course: Work under this contract is expected to be completed in FY 1980.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title	:	Endocrine Changes in Vasectomized Men
Contract Number	:	NO1-HD-2-2776
Contractor	:	University of Texas Medical School at Houston
Money Allocated	:	$85,553 (FY 1978 funds)

Objectives: The purpose of this study was to determine whether vasectomy has adverse effects on endocrine function as judged by circulating levels of gonadotrophic and gonadal hormones.

Major Findings: Plasma levels of FSH, LH, testosterone, estradiol, and 20α-dihydroprogesterone were measured in 200 men on two occasions before vasectomy, on the day of surgery, and 1 and 6 weeks and 3, 6, 12 and 24 months postoperatively. In addition, 3-year samples were obtained in 120 of these men. Mean values for all hormones at all time periods were well within the normal adult male range. There were no statistically significant differences over time in mean values for FSH and 20α-dihydroprogesterone, while estradiol levels demonstrated a slight decline and LH levels a gradual increase. Early reports of testosterone data indicated a statistically significant increase within the normal range starting at 6 months and continuing through the two year specimens, but this increase (which is the reverse of what would be expected if vasectomy resulted in testicular damage) disappeared almost entirely when the samples were re-tested with all specimens from a single patient run in the same assay.

Significance to Biomedical Research and the Program of the Institute: This study has provided longitudinal data on the effects of vasectomy on the major reproductive hormones in a large series of patients followed for 2-3 years. The data provide no evidence for physiologically significant changes in any of these hormones. The study also has demonstrated the need for careful consideration of problems of assay drift when designing longitudinal studies of endocrine function. This problem becomes of increasing importance since the widespread use of radioimmunoassays now makes such studies feasible.

Proposed Course: Work under this project has been completed.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title : Immunopathologic Consequences of Vasectomy
Contract Number : NO1-HD-3-2717
Contractor : University of New Mexico
Money Allocated : $126,174

Objectives: This contract supports studies of immunopathologic effects of vasectomy in experimental animals and man. In vasectomized men, the investigations are directed to defining the class and antigenic specificity of circulating antibodies to sperm antigens, the development of cell-mediated immunity, and the detection of circulating immune complexes. In vasectomized animals and animals with experimental allergic orchitis (EAO), pathological changes in reproductive and other organs are being evaluated and efforts are being made to define the roles of specific sperm antigens, T and B cells, and tolerance or the lack thereof, in the development of pathology.

Major Findings: Substantial purification of aspermatogenic antigen preparations from guinea pig sperm has been achieved, yielding three fractions which consistently induce EAO in guinea pigs when injected with complete Freund's adjuvant at doses of 0.2 µg, and one fraction which is consistently active at 2 µg. Further purification is in progress, and these preparations are also being used in studies of antigen-mediated suppression of EAO and of cell-mediated immune responses to sperm. Previous studies of cell-mediated immunity have been open to question because they have used intact sperm and so may have measured responses inhibited by a known suppressor factor in seminal plasma or induced by irrelevant histocompatibility antigens or sperm enzymes. The partially purified fractions are free of these complicating factors. Recent data demonstrate that guinea pigs with EAO do develop cell-mediated immune responses to one of the partially purified aspermatogenic antigens.

Significance to Biomedical Research and the Program of the Institute: Vasectomy in rabbits leads to an immune complex disease of the testis involving deposition of complexes, destruction of the seminiferous epithelium and sometimes glomerulonephritis. In guinea pigs, and apparently in monkeys, immune complexes are not seen in the testis but there is a diffuse or focal monocytic orchitis with areas of hypo- or aspermatogenesis. The nature of this disease needs to be determined in relation to possible sequelae of vasectomy in man and the lack of success in reversal of vasectomy.

Proposed Course: Work under this contract is expected to be completed next year, with further studies of post-vasectomy orchitis being pursued through a new solicitation of proposals.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title : Long-Term Effects of Vasectomy in the Rhesus Monkey
Contract Number : NO1-HD-4-2866
Contractor : Oregon Regional Primate Research Center
Money Allocated : $27,713

Objectives: The purpose of this contract has been to evaluate the effects of vasectomy in rhesus monkeys which were vasectomized up to 14 years ago. Parameters studied include general health status, hormone levels, humoral and cell mediated immune responses to sperm antigens, autoantibodies to non-genital antigens, and complete pathologic and immunopathologic assessment at autopsy.

Major Findings: Autopsies of the 36 vasectomized and control animals have been completed during the past year, with significant findings in two areas. First, the exacerbation of atherosclerosis in vasectomized animals, previously reported by these investigators in cynomolgous monkeys fed a high cholesterol diet for 10 months (see contract NO1-HD-8-2827), has been confirmed in this study of rhesus monkeys fed normal monkey chow, which is cholesterol-free and very low in fat. Significant increases in the extent and severity of atherosclerosis were demonstrated in the major arteries of 10 monkeys vasectomized 9-14 years before sacrifice, as compared with 7 age-matched controls. In the second area, somewhat equivocal data suggest an increase in immunologically mediated disease of the testis and these studies are being extended to tissues from ten additional animals, 5 vasectomized and 5 controls, in an effort to clarify the nature of this pathology and its relation to vasectomy.

Significance to Biomedical Research and the Program of the Institute: This project has offered a unique opportunity to evaluate the long-term effects of vasectomy in a subhuman primate. Most of the findings, except the consistent demonstration of antisperm antibodies, have been reassuringly negative. The finding of increased atherosclerosis in vasectomized monkeys is unquestionable and has stimulated the initiation of studies to determine whether or not the relationship applies in man.

Proposed Course: Pathologic evaluation of testes and epididymides from the ten additional animals is expected to be completed this year, and work under this contract will then be completed. Studies to evaluate the relationship between vasectomy and atherosclerosis in man have been solicited and one or more new contracts are expected to be initiated in FY 1980.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title : Immunologic Studies of Vasectomy
Contract Number : NO1-HD-5-2841
Contractor : Wayne State University
Money Allocated : $140,028 (FY 1978 funds)

Objectives: This contract supported prospective clinical studies of vasectomized men with and without a genetic predisposition to autoimmune disease. Parameters measured include health status, circulating antibodies to sperm and to non-genital tissue antigens, and circulating immune complexes.

Major Findings: One hundred ninety-four men have been studied before vasectomy and at 6 weeks and 1 and 2 years after vasectomy. These include 73 men with a positive personal or family history of autoimmunity, 69 with questionable histories, and 52 with no history of autoimmune diseases. Patients with a history of autoimmune disease demonstrated a slightly greater frequency and higher titer of postoperative antisperm antibodies, as did men vasectomized at younger ages. No differences were seen in the incidence of autoimmune disease which might have been stimulated by the immunologic response to vasectomy.

Significance to Biomedical Research and the Program of the Institute: Although a substantial proportion of vasectomized men develop an immune reaction to sperm antigens which could lead to a variety of immunologic disorders, no serious sequelae have as yet been identified in vasectomized men. Vasectomy patients who are genetically predisposed to autoimmune diseases might be expected to demonstrate an increased frequency of immunological disease after surgery, but no such effect was demonstrated during the period of follow-up in this contract.

Proposed Course: Work under this contract has been completed.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title : Immunologic Studies of Vasectomy
Contract Number : NO1-HD-5-2842
Contractor : University of Connecticut Health Center
Money Allocated : $121,769

Objectives: This contract supports immunopathologic investigations of the effects of vasectomy, particularly the development of immune responses to sperm antigens and the incidence and course of immune complex orchitis in laboratory animals.

Major Findings: Studies of vasectomized and control animals of several species and strains are in progress. In specific-pathogen-free and normal rabbits, anti-sperm antibodies were demonstrated in 83% of the vasectomized animals but in none of the sham-operated animals or pre-vasectomy sera. Antibodies were not detectable until 13 months or more after surgery. Immunoglobulin deposits have been demonstrated in the testes of more than two-thirds of the vasectomized rabbits, and they also occur in the epididymis in a few animals. Further pathologic and immunopathologic evaluation of these specimens is in progress, as are studies of vasectomized and control rats and mice of several genetic strains and human biopsy tissue as it becomes available.

Significance to Biomedical Research and the Program of the Institute: Immune complex orchitis was first reported by this contractor in vasectomized rabbits. This finding has now been confirmed by other investigators and other types of post-vasectomy orchitis, involving lymphocytic invasion but not immune complex deposition, apparently occur in guinea pigs and monkeys at long intervals after vasectomy. Studies under this contract will help define the range of pathology and some features of the pathogenetic mechanisms involved in various species of laboratory animals.

Proposed Course: Work under this contract is expected to require at least another year.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Health Status of American Men

Contract Number	Contractor	Money Allocated
NO1-HD-6-2807	University of California at Los Angeles	$312,103
NO1-HD-7-2801	Mayo Clinic	207,000
NO1-HD-7-2802	University of Southern California	569,928
NO1-HD-7-2803	University of Minnesota	414,049

Objectives: The purpose of this collaborative study is to identify long-term health hazards which may be associated with vasectomy as a contraceptive procedure. The current health status and medical histories of previously vasectomized men are to be compared with those of non-vasectomized control groups. The contracts with the Mayo Clinic, USC and the University of Minnesota provide for data collection according to a common protocol, and the contract with UCLA provides for project coordination and data analysis.

Major Findings: In the first phase of this study, a detailed protocol and manual of procedures was developed and subjects for the study were identified. The study is now in the data collection phase and information has been obtained and processed on over 10,000 subjects. Plans for analysis are well under way but analyses of possible differences between cases and controls have been deferred pending completion of data collection in order to avoid any possibility of introducing bias into the collection of the remaining data.

Significance to Biomedical Research and the Program of the Institute: Vasectomy is an increasingly popular method of contraception which has generally been considered to have no adverse effects. However, it is known that a substantial proportion of vasectomized individuals develop circulating antibodies to spermatozoa and that, in rabbits, guinea pigs, and perhaps monkeys, this immune response can result in disease in the testis and sometimes kidney. Furthermore, in monkeys there is now strong evidence that vasectomy leads to exacerbation of atherosclerosis, presumably through immunologic injury to arterial walls. This study will be able to identify differences in incidence of common and also quite rare diseases in vasectomized men as compared to controls, or to demonstrate that there is no increase in risk within certain defined limits of probability.

Proposed Course: Data collection is expected to be completed in another 2-2½ years, and final analyses of the data will require a further 6 months.

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Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title	:	Effect of Bilateral Vasectomy on the Progression and Regression of Atherosclerosis in Macaca fascicularis
Contract Number	:	NO1-HD-8-2827
Contractor	:	Oregon Regional Primate Research Center and Bowman Gray School of Medicine
Money Allocated	:	$115,529

Objectives: The purpose of this contract is to pursue preliminary findings by the contractors which indicate that vasectomy may significantly increase the severity of atherosclerosis in monkeys fed a high cholesterol diet. The effects of vasectomy on atherogenesis will be studied in normocholesterolemic and hypercholesterolemic monkeys fed a diet comparable in cholesterol content to that of the average American man. The possible therapeutic role(s) of a more prudent diet and/or vas reanastomosis on regression of established atherosclerosis will also be evaluated.

Major Findings: Sixty cynomolgous monkeys have been selected for study at Bowman Gray and 75 at Oregon. The animals have been acclimated to the experimental diet for 6 months, and half of those at Bowman Gray and all of those at Oregon have been vasectomized. They will now be followed, with periodic determinations of blood lipids, blood pressure, sperm antibodies, and other parameters, for a period of 18 months prior to sacrifice for evaluation of the extent of atherosclerosis or the start of regression studies.

Significance to Biomedical Research and the Program of the Institute: Vasectomy is increasingly popular as a method of permanent contraception and has been used by millions of American men. No serious long-term medical consequences of the procedure have been identified but it is established that half to two-thirds of these men develop antisperm antibodies after surgery. These antibodies may circulate as complexes and there is a body of evidence suggesting that circulating immune complexes exacerbate the atherogenic process. If the contractor's preliminary findings in monkeys are confirmed and are applicable to human beings, they would suggest that vasectomized men with high cholesterol levels may be at high risk for cardiovascular diseases attributable to atherosclerosis.

Proposed Course: The animals in this study will be maintained on the experimental diet for 18 months. At that time, the monkeys at Bowman Gray will be sacrificed and all major arteries evaluated for amount of atherosclerosis. Fifteen of the monkeys at Oregon will also be sacrificed at 18 months after surgery and similarly evaluated. The remaining monkeys at Oregon will be used in studies to determine the value of vas reanastomosis and/or dietary restriction in regression of established atherosclerosis.

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Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title:	Survey of Womens' Health and Birth Control Methods	
Contract Number:	NO1-HD-5-2852	$215,419
	NO1-HD-7-2811	0
	NO1-HD-6-2806	0
	NO1-HD-7-2807	0
	NO1-HD-6-2804	0
	NO1-HD-7-2808	0
	NO1-HD-7-2809	0
	NO1-HD-7-2812	0
	NO1-HD-6-2805	0
	NO1-HD-7-2810	0
Contractors:	Biostatistics Center of George Washington University, and nine clinical centers	
Money Allocated:	$215,419	

Objectives: These contractors are collaborating to conduct a multi-center case-control study of the relationship between IUD use and the occurrence of seven serious gynecological and obstetrical disorders (uterine perforation, pelvic inflammatory disease, septic and non-septic fetal loss, vaginal hemorrhage, ectopic pregnancy, abruptio placenta, and placenta previa). The analysis of the data from the study is directed towards estimating the effect of past and current IUD use on the risk of development of each of these seven disorders, both in general and according to specific brands of IUDs used. This information should be of considerable value in clarifying the health effects of IUD use.

Major Findings: Using a standardized Study Protocol, Manual of Field Procedures, and set of data forms, data have been gathered in 9 clinical data collection centers on 8,851 cases (all diagnoses) and 10,371 controls. These data include demographic characteristics, medical, surgical, and reproductive history, and birth control history. The data have been computerized at the study coordination and data processing center. Four report preparation committees are in the process of analyzing the data and preparing reports of findings.

Significance to Biomedical Research and Program of the Institute: An increased risk of pelvic infection and other gynecologic disorders among IUD users has been reported in the literature. The current study is much larger and more detailed than the previously reported studies. This will allow analyses aimed at characterizing women at especially high risk for complications of IUD use, and will also allow comparisons of risks for the different IUDs currently in use.

Proposed Course: Data collection has terminated and data analysis is in progress. It is anticipated that this project will terminate in the Spring of 1980.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract: A Prospective Study of the Effects of Induced Abortion on Subsequent Reproductive Function
Contract Number: NO1-HD-6-2802
Contractor: New York State Health Department
Money Allocated: 0

Objectives: To evaluate the effects of induced abortion on subsequent reproductive function by linkage of abortion registry data to vital statistics.

Major Findings: This project commenced September 1, 1975. The basis for the study is the registry of 27,987 induced abortions recorded in upstate New York between July 1, 1970 and June 30, 1971. Women with recorded induced abortions are being compared to women with recorded live births concerning the outcome of subsequent pregnancies.

The exposed (abortee) and unexposed (live birth) women's index records have been linked with their records of subsequent live births and fetal deaths. Linkage through the registry of marriages has been performed to account for name changes.

A sub-sample of the exposed and unexposed women has been studied via the motor vehicles department registry of drivers' licenses to estimate whether loss to follow-up has been different for the exposed and unexposed groups.

Establishment of the entire computerized data base is complete. Data analysis is in progress. The preliminary analyses suggest an increased risk of adverse pregnancy outcomes among women with prior induced abortions.

Significance to Biomedical Research and Program of the Institute: This very large data linkage project will complement the other 2 projects in this program area. Taken together, the three projects should increase knowledge concerning the effects of current induced abortion practices on subsequent reproductive function.

Proposed Course: A site visit was held in June, 1979 to establish the scope of the analyses to be performed. The project is now scheduled for completion and submission of a final report in March, 1980.

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Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Prospective Study of the Outcome of Pregnancy Following Induced Abortion
Contract Number: NO1-HD-6-2803
Contractor: State University of New York
Money Allocated: 0

Objectives: To evaluate the effect of single and repeated induced abortion on the subsequent reproductive function of nulliparous women.

Major Findings: The study commenced October 1, 1976. Initial work involved elaboration of the study protocol, establishment of arrangements with appropriate prenatal clinics, and the development and field testing of the study data forms.

The study is now operational and data acquisition is proceeding in the prenatal clinics of several hospitals and in several private practices.

Procedures for data management and analysis have been established.

Significance to Biomedical Research and Program of the Institute: The effects of induced abortion on subsequent reproductive function are of substantial practical importance, given the widespread practice of induced abortion. This project is expected to yield detailed information about the effect of single and repeated induced abortion on the subsequent reproductive function of nulliparous women, and should thus complement the two more broadly focussed record linkage projects in this program area.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Effects of Induced Abortion on Subsequent Reproductive Function and Pregnancy Outcome in Hawaii
Contract Number: NO1-HD-7-2801
Contractor: University of Hawaii at Manoa
Money Allocated: $118,359

Objectives: To evaluate the effects of induced abortion on subsequent reproductive function by linkage of state abortion records with live birth and fetal statistics. Specifically, to study the effects of induced abortion upon rates of subsequent spontaneous abortion, premature birth, and other birth-related disorders.

Major Findings: Pregnancy outcomes which occurred between January, 1971 and December, 1976 are being compared for 16,500 women who collectively underwent 17,702 recorded induced abortions between July, 1970 and June, 1974 and two control groups: 1) all live births and fetal deaths which occurred in the State of Hawaii between January, 1971 and December, 1976 minus those which occurred to women in the "recorded induced abortion" group; 2) all live births and fetal deaths which occurred between January, 1971 and December, 1976 to women without a prior recorded induced abortion who are matched to the women with such a history for maternal age, racial background, residence (urban vs. rural), and birth order.

The preliminary analyses of these comparisons suggest a higher rate of spontaneous abortion and fetal death among women with prior recorded induced abortions than among women with no prior recorded induced abortions.

A follow-up study based on a sample of the total cohort study subjects is being developed to evaluate whether loss to follow-up varied according to prior abortion history. Also, data on important potential confounding factors such as cigarette smoking will be gathered by direct interview of the subjects selected (by random sampling) for the follow-up study.

Significance to Biomedical Research and Program of the Institute: This data linkage project provides a large data base from which to estimate the effects of induced abortion on subsequent reproductive function. It complements the other data linkage project and the cohort study which constitute the elements of this program.

Proposed Course: This contract has been extended to September 21, 1980 to allow completion of the follow-up study.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Center for Population Research

The Center's grant and contract program in the behavioral-social sciences focuses on the size, rate of growth and composition of our nation's most important asset, its population. The topics addressed by specific research projects derive from fundamental questions concerning the growth and distribution of our population, and their relation to individual and societal welfare. For example: What accounts for changes in the number of children couples decide to have? How successful are they in controlling their reproduction? What are the consequences of many or few children for individuals, families, and the nation? What are the effects of age at birth and birth spacing on mothers and their children? How does internal migration affect the welfare of the people moving and the communities from which they come and to which they go? How would a rise or decline in the rate of population growth rates from a combination of natural increase and net immigration affect our economy and our environment in the future? Answers to these and many other questions are needed in order to deal with population changes now and in the years ahead. The results of research are intended ultimately to help individuals and couples to understand the personal and societal consequences of their behavior and to help the government evaluate the impact of a variety of policies and programs on both the quantity and quality of our human resources.

The staff of the Social and Behavioral Sciences Branch uses grants to support research on these broad objectives, and uses contracts to support research on more specific research goals identified by the Institute. The Branch's strategy has been to concentrate on topics of current societal importance as well as questions of scientific concern. Consequently, the program has emphasized not only such topics as social and economic factors affecting the level and trend in national birth rates, but also such topics as the methods of fertility control used, effectiveness of contraceptive practice, changes in family size over time, the consequences of family size, the determinants and consequences of adolescent fertility, and the implications of immigration for the U.S.

One result of this strategy is that the Branch's research program changes in response to changing social conditions as well as in response to developing scientific knowledge. At present, its program focuses on the topics listed below:

Trends and Variations in Fertility

The series of national fertility studies begun in 1955 have detailed the trends in family building intentions and the practice of contraception among married women. The 1975 study shows that the number of births regarded as ideal, desired, and intended all declined substantially between 1970 and 1975. Improved control of fertility has reduced the incidence of unintended births, and sterilization is becoming the major method of limiting family

size in the United States--an estimated two-thirds to three-quarters of once-married white couples will obtain sterilizing operations within 15 years after their last wanted birth. These factors help to explain the current low level of fertility in the U.S. One fertility study gathered data on the cohort of women born 1901-1910, since they had the lowest completed fertility in American history (an average of 2.3 births per woman). This study was undertaken to clarify the extent to which the present low fertility is uniquely tied to a definite historical period or is a response to more general kinds of social and economic changes. Seventy percent of the women reported that they or their husbands had used some method of contraception. The most commonly used method was the condom, followed by douche and withdrawal. Among the women who did not use a method to prevent pregnancy, 57 percent reported douching for cleanliness. In addition, one-fourth reported having had an operation that caused them to be sterile before the end of the reproductive period, although many of these operations were not specifically for contraceptive purposes. Initial results also indicate that couples most severely affected by the economic depression had more children than those less affected--partly because those most affected were a high-fertility group.

To supplement these analyses, research has also been focused on the social and economic explanation of the extraordinary rise in fertility that occurred in the U.S. after 1940, and its subsequent decline since about 1958. Professor Richard Easterlin hypothesized that the rise and decline can be attributed to changes in perceived economic opportunities for persons in their early adult years relative to the aspiration levels established in their youth. When these opportunities are seen as relatively high, earlier marriages and childbearing are given more encouragement than when they are not so viewed. Research designed to test the validity of this hypothesis has produced mixed results. Generally, the macroanalyses using vital statistics, census data and econometric models for the period 1940-1974 have given strong support to the main components of the Easterlin hypothesis. Cohort analysis suggests that the effect of relative income on cohort fertility is positive and significant. Increases in a cohort's expected lifetime income induces earlier childbearing, but increases in the parental generation's lifetime earnings has no significant effect on the timing of its offspring's fertility. However, microdata collected 1955-1970 have not supported the Easterlin hypothesis. These data were more consistent with the hypothesis that aspirations--particularly those not related to household consumption--are negatively related to fertility.

National fertility survey data were also examined regarding female labor force participation, education, concern about population growth, and the age structure. It appears that the baby "boom" occurred in spite of changes in these variables and not because of them. With the possible exception of female labor force participation, all were moving in directions which would be expected to lower fertility. However, female labor force participation, and to a lesser extent each of the other variables except age structure, could account for only part of the baby "bust".

Factors Affecting the Control of Fertility

The Center continues to stress the importance of broadening and deepening knowledge about and understanding of the factors that are involved in the regulation and control of fertility. Several studies approach this problem by attempting to identify and analyze psychosocial factors which enter into the making of decisions to have children. Thus, preliminary findings show that spouses who disagreed regarding number and spacing of children, when compared with those agreeing, (a) desired a larger family size, (b) discussed family size and sexuality less frequently, and (c) communicate feelings less frequently. For recently married couples, disagreement regarding fertility was associated with full-time employment, high education and high socio-economic status of wife. Only about three-quarters of wives and husbands were accurate in their perceptions regarding agreement of the couples on current family size desires. Where husbands stated they disagreed with their wives, they thought their wives wanted more children than they actually wanted. These findings suggest that encouragement of greater openness and discussion among spouses, perhaps by educational programs, counseling, and other means, might lead to clearer bases for choice of desired family size, and for ways of achieving such a size.

Another project has attempted to understand various aspects of reproductive behavior, such as reproductive intentions, contraceptive practices, and child-bearing, in terms of the anticipated consequences of having children. Among the areas of consequences which were studied, married couples' concern about the financial costs of bearing and rearing children was a fairly consistent predictor of contraceptive effectiveness and reproductive intentions. Preliminary findings from a different study are that perceived benefits are more important for family size preferences than perceived costs or such measures of economic status as income per adult, perceived income adequacy, or type of employment. Each of these studies helps to explicate how the anticipation of what having children will be like influences fertility control practices and desired fertility. The importance of the sex of a child and the way a wife would feel about herself if she were to become pregnant in the near future were other areas of consequence which were frequently significant predictors of reproductive behavior and events. But another investigation has demonstrated that most people make distorted estimates, based upon incorrect reasoning, of the probable sex of the next child in a family.

A model has been proposed to relate married couples' anticipated consequences of having children with reproductive behavior, and has shown that using the consequences anticipated by the married couple resulted in greater predictive power than single-sex, e.g. female only, models. Another methodological advance has been made in the study of family size preferences. One study has indicated that the probability of having an unwanted birth was greater than average for those with an underlying preference for larger families. Other studies focus on motivational factors associated with subgroups of the population.

One investigator reports that while most coitally active college students use some form of contraception regularly, sizeable numbers are not protected. It is shown that being in love with one's partner increases the use of contraceptives, while less use occurs for those in a non-love relationship. In addition, college women exhibit a relatively high degree of dissatisfaction with the pill. Considering that both the nature of the relationship of the sex partners and of the contraceptive are important, many college women may be at high risk for pregnancy.

Each year over one million women in the United States control their fertility by means of legal abortions. Two studies are analyzing abortion as a social issue. Attitudes toward making legal abortion available became much more favorable between 1965 and 1973, and did not change until 1978, when there was a small but significant decrease in approval of abortion for psychosocial reasons. Analysis of conditions under which people regard abortion as justified (mother's health, birth deformity, economic stress, and elective abortion) reveals that the most important distinction is between whether people disapprove of abortion for all reasons and not whether they express approval or disapproval according to type of justification. Education is the socioeconomic status indicator most strongly correlated with abortion attitude, approval of abortion increasing with the amount of formal education. A slight tendency was found for political conservatism to be correlated with disapproval of legal abortion, especially among people with some college education. A study of obstetrician-gynecologists in one state indicates that forty-three percent do no abortions and 60 percent do no midtrimester abortions. Religion is the most important variable accounting for the performance of abortions. Only fifteen percent of Catholic physicians currently perform abortions as compared with seventy-eight percent of physicians with other religious affiliations. Urban residents, group practitioners, and board certified obstetrician-gynecologists are more likely to perform abortions. Seventy-seven percent of the ob-gyns favor the 1973 Supreme Court decision; ninety-two percent of the "favorable" group had done an abortion. Of the physicians with abortion experience forty-two percent reported being disturbed by the procedure. These "disturbed" physicians were more likely to ask women to obtain consent from spouse or parent, and were more likely to charge lower fees.

Consequences of Adolescent Pregnancy and Childbearing

Concern about early pregnancy and childbearing revolves around the effects on the young woman, her child, the father, and other family members involved, as well as society as a whole. While birth rates of older adolescents have declined in recent years, birth rates of middle adolescents have remained fairly high and the rates have increased among younger adolescents. There has been an increase in the likelihood of out-of-wedlock births and adolescents still account for one third of the legal abortions performed in this country each year. There is substantial evidence of high levels of unintended pregnancy and childbearing among adolescents and research is generating an improved specification of the effects of this behavior.

Early childbearing has a wide variety of effects on the mother. First, there is a substantial impact on her educational attainment. Even when previous interest or aptitude is taken into account, an early first birth has a negative effect on completed schooling. While many young mothers go back to school after the birth, longitudinal studies show that they are not able to catch up to those who did not experience an early first birth. Similarly, there is a consistent and strong relationship between early motherhood and later economic distress, largely as a function of reduced educational attainment.

The economic position of these women and their children is also influenced by their fertility and marital histories. Several studies have documented the effect of an early first birth on subsequent fertility showing that the woman who begins her childbearing as an adolescent is more likely to have a larger number of children, more unwanted births, more out-of-wedlock births and a faster pace of subsequent childbearing. An early marriage is associated with increased risk of marital dissolution, although the evidence is less clear as to whether the presence of a child is an added disadvantage. Since pregnancy often precipitates these marriages, the association at least is clear. This risk of marital dissolution is carried on through later life and shows up in increased risks of marital dissolution in second marriages. For the adolescent mother who is not married, studies show that she is very likely to marry soon after the birth, but she, too, is at high risk of divorce. These studies present a consistent picture of the adolescent mother as experiencing a constellation of effects of her early entry into motherhood. Her education is interrupted and her higher fertility impedes her development of job skills and work experience. Her marital history is such that there is often no male able to provide for her and her children's needs and they are at increased risk of welfare dependency or low economic status, if the family depends on the woman's earning ability. Not surprisingly, studies of the mothers' reactions to their early entry into motherhood show increased levels of reported stress, marital and occupational regrets and regrets over the timing of their first birth.

Recent studies point to the importance of studying the interrelationship of the adolescent mother and her family. Studies have found that unmarried adolescents were highly dependent on their families in the first several years after the birth. Factors influencing the amount of aid were the adolescent's desire to finish school, her emotional reliance on her parents, the family's economic resources, available space in the household and the adolescent's additional childbearing. The adolescents who did receive such aid were more likely to complete school. The teenage birth conveys both burdens and benefits on the family and that balance varies from one family to another. Preliminary analyses show that the families do not experience disadvantages in their own socioeconomic and family careers as a result of the teenagers' births.

A number of projects are looking at the consequences of adolescent childbearing for the children involved. Several studies have examined the effects of maternal age on pregnancy complications and the resulting risk to the newborn. These studies suggest that the negative effects of

maternal age and the social factors on pregnancy and neonatal health previously reported were largely mediated by the quality of health care received by the mother and infant rather than being a function of the mother's biological age.

Studies examining the child's later development have shown that mother's age at child's birth and social factors are related to the child's subsequent physical health, cognitive and social development. One study using measurements taken at one year of age found that children of low SES and children of unmarried mothers who live alone with their children generally show poorer physical health. In addition, children of older mothers, 25 years and up, are healthier than children of younger mothers, except in cases where teenage mothers rely upon older women (i.e., grandmothers) for child care. Another study found differences in mother-infant interaction patterns between younger and older mothers. Older mothers both vocalized to and looked at their babies more than younger mothers. Increased talking and looking time were associated with higher scores on tests of motor and mental skills, with more positive maternal attitudes and with more positive perceptions of the baby's temperament.

The social, emotional, and intellectual development past infancy of the children of adolescents continues to be related to mother's age at birth. Two studies have found a consistent tendency for children of adolescents to have slightly lower IQ scores than children of older mothers when measured at several ages up to 7 years and some effects of maternal age on social and emotional development have also been found. A study assessing children's social adaptation at school found a considerable risk of poor social adaptation for the children of teenage mothers, a risk that continued from the first grade through the sixteen years of the follow-up. A study of the children of adolescent mothers has found that they experience a greater probability of living in a non-intact home while in high school, have lower academic aptitude as a teenager and are at slightly increased risk of repeating the parents' pattern of early marriage, early parenthood and greater fertility. A separate study found the best predictor of a woman's age at first birth was her mother's age at first birth. The effect on academic aptitude appeared to be partly brought about by the family environment, i.e., more time spent in a single-parent, step-parent, or no-parent household. This is consistent with findings from a variety of studies which point to the importance of family structure as an intervening variable between adolescent childbearing and the effects on the mother and child.

Determinants of Adolescent Pregnancy and Childbearing

A wide range of studies are addressing the factors associated with adolescent sexual, contraceptive and fertility behavior. Many innovative methodological techniques are being used. Family structure, which appears influential in translating the effects of early parenthood, also plays an antecedent role. An analysis of national data suggests that family structure variables are significant predictors of the probability of intercourse for adolescent women. Teenage women who grow up in a household with both a male and

female parent are less likely to be sexually experienced than adolescents in a single parent household, after controlling for age, religion, and various other socioeconomic characteristics. Educational expectations have a significant negative effect on the probability of intercourse, as do father's education and the age of the household head. Teenage women who work and go to school are less likely to be sexually experienced, but among the subsample of sexually active adolescents, work and school appears to be associated with a younger age at first intercourse. Another study focused on psychosocial determinants of adolescent contraceptive practice and found various liberal attitudes about sex were powerful differentiators of virgins from non-virgins. The non-virgins appeared more self confident, saw contraception as more available, were aware of the need for its use and were more traditional in their views of male-female relationships. The virgins saw more negative features of contraception and were more embarrassed about the idea of discussing it. A study of interpersonal skills training gives some evidence that changes can be affected in self esteem and feelings of control. A study of mother-daughter communication showed better contraceptive practices for adolescents when there was communication between mother and daughter but also showed woefully low estimates of daughters' sexual activity by mothers. Preliminary analyses from a Chicago based study have shown that, especially among younger adolescents (ages 13-15), facts about reproductive anatomy and physiology, thoughts and experience with conception, contraception, and heterosexuality appears as pieces of a complex puzzle that do not fit together and are only dimly perceived. Females are far more concerned and knowledgeable about conception and contraception than are male partners, yet their effective use of contraceptive methods is compromised by incomplete understanding of how they work and by notions that certain methods will not work for them. The difficulty which some experience with defining the nature and beginning of their menstrual periods affects their use of oral contraceptives and the early determination of pregnancy.

A comparison of national data from surveys done in 1971 and 1976 shows that the use of effective methods of contraception has increased among unmarried teenage girls, but there are still many who do not use contraception. The major reason given for not using contraception is the belief that pregnancy could not occur--for a variety of reasons, including having intercourse at the time of the month when it was thought that pregnancy could not occur, being too young to become pregnant, and having intercourse too infrequently to become pregnant. Another important reason for not using contraception was that the girl had not expected to have intercourse.

In addition to these studies of personal and interpersonal factors, other studies are looking at macrolevel conditions that may be related to adolescent sexual, contraceptive and fertility behavior. These projects will address how policies regarding contraceptive services, welfare policies, and unemployment rates are related to levels of adolescent fertility-related behavior.

Consequences of Family Size and Population Growth

As several studies cited above have indicated, the anticipated consequences of having a child or an additional child is an important factor affecting fertility. Because couples planning their childbearing patterns as well as those who counsel them and provide health services need more information on these effects, research emphasis in recent years has also been on the consequences of childbearing and childspacing for both parents and children. Three recently completed studies dealt with the effects of family size on child development and achievement. One studied the effect of age at marriage and time of first birth on family interrelationships, children's personality and cognitive development. This study found first borns of late marriages highest in general intelligence, verbal ability and self-esteem while being less ego defensive. Parents who spaced their children more widely had more desirable psychological characteristics than parents who did not. Concerning family interraction, the study found that first borns from early marriages were more non-compliant with maternal commands than other children and that mothers in early marriages issued more maternal commands to first borns than did other mothers. Another study analyzed the effects of birth order and sex of infants on mother-child interaction and on the cognitive and emotional development of infants. Unique findings from this study show that birth order, spacing and sex of the child have affected mother-infant relationships and the child's cognitive performance as early as three months into the child's life. Many of these effects found at three months are still evident at 12, 24 and 36 months of age.

A major program initiative is underway on the consequences of low fertility, more specifically the effects of childlessness and of one-child families on the adults and children involved. These effects may differ by age, either of the parents or of the child, and according to other characteristics such as sex of the child, intendedness of the family size and so forth. Therefore, a large variety of projects are necessary to give readings on this phenomena at different points in time and under different conditions. Also, the effects may cover a wide range of factors from psychological functioning to economic attainment. Upon the completion of these studies, understanding of the effects of childlessness and one-child families will be greatly enhanced.

Some studies specifically focusing on the consequences of having or being an only child will address a wide range of outcomes and data will be collected from subjects drawn from different age groups and settings. Special attention will be paid to factors which may mediate the effects of family size. One study is using extant data on children, their mothers and mother-child interaction at 3, 12, 24 and 36 months of age for the child. Longitudinal and cross-sectional analyses contrast behaviors and cognitive levels of only children and first borns. This study measures the impact of acquiring a sibling at two ages, and contrasts planned and unplanned children.

Preliminary findings based on measures taken when all infants are only children, show differences in performance on standard IQ tests to indicate

that those who will have siblings have higher IQ's than those who will not have siblings. Since the role of the sibling cannot be a factor (no sibling is yet born), these results suggest that maternal differences in caregiving may be responsible for the differences in the children's performance. Preliminary observations of maternal data appears to support this conclusion.

New research on the consequences of childlessness cover a wide range of outcomes. Six studies are looking at the effects on men, women and married couples at different points in the life cycle and are assessing the relationship of childlessness to educational and occupational attainment, indicators of social and economic well-being, and measures of personal and marital satisfaction. The voluntary or involuntary nature of childlessness will be taken into account wherever possible. Different studies are looking at the consequences of 0 and 1 child families as compared with families with more children. Through the analysis of available data, the relationship of family size with social adjustment, psychological well-being, perceived quality of life, physical health, occupation, education, marital stability and other factors will be assessed. Some will focus on the children and others on the parents. One study in this area of interest deals with comparative levels and trends in childlessness and one-child families in the U.S. and around the world. Preliminary findings for the U.S. show that rates of childlessness are changing so that the differential between blacks and whites is disappearing. Previously, rates of childlessness among blacks were nearly double that of whites. This study also shows that, on the basis of fertility rate changes between 1960 and 1974, one-child families in the U.S. are increasing. Another study uses national survey data over a period of about 35 years to measure the general motivation for below-average reproductive behavior, and the general climate of opinion concerning childlessness and one-child families. Preliminary findings are that childlessness remains a non-preferred way of life, although there is considerable disagreement as to the disadvantages. There is some increase in the belief that childlessness is viewed less negatively among women, the highly educated, the divorced and single, the young, the urban, the well-to-do, and non-Southerners. However, even within the above groups, only very small percentages view childlessness as an advantageous condition.

Two projects dealing with the economic and environmental consequences of population growth were completed in FY 79. One project has produced a computer simulation model of population, resources and environment. Preliminary tests of the model indicate that declining levels of population are preferable to positive rates of population growth. Another study addressed the relationship of economic growth and depletion of resources with changes in population growth, over the next 25-50 years. The importance of population in causing resource and environmental pressures was found to depend on the time period one chooses to frame the study, with the impact growing as the time period lengthens. The study illustrated the adaptation that the economy must undergo to cope with a wide variety of economic, environmental and demographic circumstances.

Migration and Population Distribution

The impact of population levels, especially at the local level, is often influenced as much by migration as by fertility levels. Analysis of State Economic Area migration data identified significant numbers of migrants moving from non-Southern cities (SMSAs) to Southern SMSAs. Also, return migration is playing some part in the revival of non-metropolitan areas observed in this decade. Some return moves occur soon (1-2 years) after an initial move; others occur only after a much longer interval in the city.

Similarly, immigration to the U.S. is a substantial contributor to overall levels of growth. Presently, legal immigration accounts for one-fourth of the yearly population increase observed in the U.S. and illegal immigration is estimated to contribute even more. Current estimates of legal and illegal immigration, coupled with the present low fertility rates, would result in a U.S. population in the year 2000 very similar to that obtained from a higher level of fertility without illegal immigration. Previous NICHD supported research points to the complexity of migration research issues. A study of migrants from Mexico involved interviews in nine regions in Mexico and 77 localities in California and Illinois where the migrants were living. Despite high levels of natural increase, all nine regions were losing population due to emigration in the 1960's. Most Mexican rural development programs failed to reduce out-migration, and 41 percent of the migrants leaving the research communities in the 1960's came to the U.S., an estimated 70 percent of that movement being illegal. Moreover, population pressures seem to be increasing the U.S.-bound movement. Much illegal migration to the U.S. is temporary with the average illegal immigrant making only 2-3 short trips during his lifetime. Family migration histories indicate temporary immigrants outnumber permanent immigrants 8:1. Increasingly, these immigrants are involved in the urban commercial, construction, industrial and service sectors, rather than being concentrated in the agricultural sector. The immigrants studied make little use of social welfare programs even though nearly all had paid Social Security and half had personal income taxes withheld. The full implications of immigrant status -- either legal or illegal -- in regard to integration into U.S. culture and society and use of the health care system are unclear and are receiving increasing research attention.

A longitudinal study is focusing on a special group of immigrants, Filipino graduate students, and is addressing the causes and consequences of decisions to remain in the U.S. or return home. In recent years the Philippines have been the largest single contributor of worker-immigrants in the U.S. in professional and technical jobs. Migration and fertility are interrelated at the individual level, and another study of Filipino immigrants to the San Francsico Bay area noted that immigrants tended to reduce their fertility over what it would likely have been in the Philippines, partly as a result of reduced access to extended family and other supports.

Workshops

A workshop on Adolescent Pregnancy and Childbearing was held on January 10-12, 1979 at the Center, and included 35 investigators of CPR-supported studies

concerned with adolescent reproduction. The researchers reported on their progress and discussed their findings. Several of these studies have been completed and the final reports are available through the National Technical Information Service (NTIS).

Targeted Research

Requests for Grant Applications (RFAs)

An RFA was issued on Studies of Unwanted Pregnancy and is part of the program in adolescent pregnancy research. Three research areas addressed were: (1) unwanted pregnancy, including the problems of measuring women's attitudes toward their pregnancies and the various factors that cause them to change; (2) the decision process, in order to understand how a pregnant woman chooses among the possible outcomes of unwanted pregnancy; and (3) the effects of pregnancy outcome on a) the mother, b) the child, c) the family, and/or d) later decisions regarding subsequent pregnancies.

An RFA was issued for studies of The Implications of Sustained Low Fertility. As a result of two CPR-sponsored conferences on this topic it was recommended that more knowledge about the relationship between fertility and health, social and economic subsystems in the United States is needed to understand how to make the best use of resources under conditions of slowing population growth. The research areas that comprise the scope of this RFA include (1) the relationship of slowing population growth to the health environment; (2) the social implications of sustained low fertility; and (3) the health and economic implications of sustained low fertility.

An RFA was also issued on the Implications of International Migration for the United States, an outgrowth of a CPR-sponsored workshop held in May 1977 on "U.S. Immigration: Research Perspectives". Abstracts of these papers were included with staff testimony in the published Hearings of the House Select Committee on Population. The RFA focuses on the stock and flow of immigrants to the United States, the impact of international migration on the U.S., and adjustment or adaptation of immigrants.

Requests for Contract Proposals (RFPs)

An RFP for Research on the Societal Consequences of Adolescent Childbearing (RFP-NICHD-BS-79-9) invited proposals for studies focusing on teenage reproduction at the national, regional, or state level, with emphasis on a comprehensive review of public-sector costs. The purpose was to investigate the impact of adolescent fertility behavior on society as a whole and compare the costs of present patterns of childbearing with other possible patterns of reproductive behavior.

Research contract proposals were also solicited for studies of the direct maintenance costs of bearing and raising children in the United States, through an RFP for Research on the Economic Determinants of Fertility (RFP-NICHD-BS-79-10). Direct maintenance costs were to be estimated taking into account age of child, age of parent(s), family

size and family income. This is one of a series of studies on the cost and value of children.

Institutional Programs in Multidisciplinary Population Research

The Social and Behavioral Sciences Branch supports three kinds of Institutional Programs in Population Research: Program Projects, Population Research Centers, and Specialized Population Research Centers at major institutions in the United States. Support for these institutional programs enables coordinated, highly skilled groups of behavioral-social scientists in a variety of disciplines to organize and conduct multidisciplinary population research programs. These programs are designed to attack complex population problems that cannot be adequately studied by an individual investigator working alone.

A. Population Research Centers

Population Research Centers supported by SBSB, together with their major research areas, are located as follows: (1) University of Wisconsin at Madison-demography and human ecology; (2) University of Texas at Austin-demography of minority groups and population change and distribution; (3) The Johns Hopkins University-demography, teenage fertility, and reproductive biology; (4) Princeton University-demography, population economics and statistics; (5) University of Washington-fertility, mathematical demography, and human ecology; (6) University of Michigan-fertility, population distribution and differentiation, and economic demography; (7) University of Pennsylvania-fertility, population movement, and economic demography; (8) University of North Carolina-fertility, family planning, migration, and population statistics.

B. Specialized Population Research Centers

The SBSB is funding one Specialized Population Research Center at the Rand Corporation. The research program has two major foci: (1) the demography of families and households and (2) regional and local population changes.

Population Research Manpower Development

A. Institutional Fellowships

The NICHD awards grants for the support of institutional behavioral-social population research training programs, and for the support of behavioral-social predoctoral and postdoctoral trainees. During fiscal year 1979, the NICHD awarded population research training grants to 12 behavioral-social programs at the following institutions: Princeton University; University of Wisconsin at Madison; University of Michigan, Economic Demography; University of Michigan, Population and Human Ecology; Brown University; University of Texas at Austin; Florida State University; University of Pennsylvania; Yale University; University of Chicago; University of North Carolina, Population Studies; University of North Carolina, Population Statistics.

B. Individual Postdoctoral Fellowships

Five behavioral-social postdoctoral fellowships were awarded during fiscal 1979.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Support of Bibliographic Preparation of Population Index
Contract Number: NO1-HD-1-2138
Contractor : Princeton University
Money Allocated: $98,288 (1971); $72,984 (1973); $90,879 (1974); $99,333 (1975) $94,235 (1976); $93,089 (1976 TQ); $99,821 (1977); $110,193 (1978); $116,123 (1979)

Objectives: The purposes are to support the bibliographic preparation by Office of Population Research staff of Population Index as a quarterly journal containing bibliographic information and special articles in the population field. Printing and distribution costs are paid by other sources of funding.

Major Findings: A 1977 site visit to the Office of Population Research (OPR) reaffirmed the value of the Index, now in its 45th year, as a comprehensive guide to articles, books, and other significant materials from the world literature, including in-house publications of relevant research organizations that deal directly with population. The staff has competency in all important western languages and confers regularly with staffs of other population organizations and library systems. The excellent physical and technical resources are matched by intellectual and organizational sources at the oldest of the U.S. population centers; the quality and efficiency of the Index operation could not readily be matched elsewhere. Publication will continue in essentially its present form. The front, author, geographic, and statistical sections are computerized, and OPR is proceeding cautiously to computerize the remainder for offset reproduction. Following up 1935-1968 cumulative publication of items, computerization of citations for the years 1975-77 are now ready (with the support of the Agency for International Development) for incorporation into a new POPLINE retrieval service under the National Library of Medicine.

During 1978 Population Index included one substantive bibliographic essay on the demographic assessment of family planning programs, two institutional profiles (Netherlands Interuniversity Demographic Institute and the International Labour Organization), and bibliography of David Glass.

Significance to Biomedical Research and Program of the Institute: Used throughout the world, Population Index is indispensable to demographic research and teaching, family planning, and to approaches to economic and social development. Its circulation of 4650 includes all members of the Population Association of America and of the International Union for the Scientific Study of Population. References to health research, planning, and delivery systems make it essential to the health mission of NIH in the population sciences.

Proposed Course: This contract, initially funded in 1971, was renewed in 1978 for three years to 1981.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : The Effect of Birth Order on Mother-Child Relationships
Contract Number : NO1-HD-42803
Contractor : Educational Testing Service
Money Allocated : $320,468 (1974); $171,159 (1977)

Objectives: The Principal Investigator will study the effects of birth order, sex of infant, and socio-economic status upon mother-infant interaction and upon the cognitive-intellectual and emotional development of the infant up to two years of age. This study focuses on infants at each of four orders of birth; first, second, third, and fourth or higher. The sample will be selected so that each of these birth-order categories will contain 60 cases. Each category will be further divided into four (15 cases each) according to sex and socioeconomic status. The subjects will be visited at ages 3, 12, 24, and 40 months. The independent variables recorded at the beginning of the study are sex of infant, birth order, class (middle or lower), mother's age at first birth, number of siblings, birth interval and sex of siblings. The dependent variables, 4 standardized tests (1 socioeconomic and 3 cognitive) and 26 structured observations, will be recorded at the time of observation. Analysis will compare children at each of the observation periods as well as their development over the period of the study.

Major Findings: Findings on 3-month-old infants are that mother-infant interaction is affected by birth order and spacing; first born children receive the most attention with a decline over the other birth orders; children spaced very close (9-18 months) or very far apart (4 years or more) received more attention than those spaced 2-3 years apart; increasing birth order was associated with poorer performance on a mental maturity test. The results are believed to indicate that birth order and spacing affect both the mother-infant relationship and the child's cognitive performance from the very beginning of life. In the cognitive domain, birth order was found to affect performance on cognitive tasks at each age level (3,12,24, and 40 months) and to affect intelligence test performance at each age level.

Significance to Biomedical Research and Program of the Institute: This study will expand our knowledge of the consequences of family size, childspacing, age of mother at birth, birth order and socioeconomic status for mother-infant interaction upon the cognitive-intellectual and emotional development of the infant. Hence it is relevant to the Institute's interest in the consequences of various childbearing patterns.

Proposed Course: This contract expired 31 July 1978.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : The Consequences of Reproduction: A Utility Model of Reproductive Behavior
Contract Number : NO1-HD-42804
Contractor : University of North Carolina
Money Allocated : $455,648 (1974); $80,775 (1977)

Objectives: Testing a utility model of fertility designed to predict the probability of various reproductive events to couples assumes that individuals regulate their reproductive behavior in light of their expectations and perceptions of the consequences of reproduction for them. Completed family size is viewed as resulting from constantly changing fertility behavior in the face of changing life circumstances and changes relating to the anticipation of a child, the birth of a child, the impact that this has on future childbearing, etc. Each of these events affect the perception of subsequent anticipated costs and benefits--the utility functions--and alter behavior accordingly. A sample of urban American women under age 32 and their husbands were interviewed in a longitudinal study to determine the relationship between normative pressures and fertility planning and the ability of a model using a variety of anticipated consequences to predict aspects of reproductive behavior.

Major Findings: Normative pressures, both expected and experienced, were related to parity or birth order--pronatal pressures decreased, and antinatal pressures increased with increasing parity. Normative expectations were modest predictors of respondents' intentions and desire to have a (another) child but were weak predictors of the effectiveness of the contraceptive regimen selected. Models using married couples' anticipated consequences of having a (another) child were moderately good predictors of reproductive behavior--again better for intentions than for the contraceptive regimen being used. Sometimes parity specific models were stronger than models which treated all parities together, and the configuration of anticipated consequences shows differences by parity. Using the consequences anticipated by both the wife and the husband resulted in greater predictive power over single-sex models. As to the causal direction of the relationship between women's fertility and their labor force participation, fertility had a substantial inverse effect on subsequent work, but working had no effect on subsequent fertility. The relationship was the same for blacks and whites, and was not complicated by significant interactions.

Significance to Biomedical Research and Program of the Institute: Testing of the important hypothesis that fertility behavior is "rational" within the perceptual framework of the actors--low income and middle income alike--has important implications for policy at several levels.

Proposed Course: This research was completed in FY 79.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Age at Marriage and Time to First Birth: A Longitudinal Study of Parental Attitudes and Children's Personality
Contract Number: NO1-HD-4-2844
Contractor : College of William and Mary
Money Allocated: $123,867 (1974); $31,668 (1976)

Objectives: This is a longitudinal study of the effects of age at marriage and time to first birth on parental attitudes, children's personality development, and family interrelationships. The major focus of the research is upon first-born children within self-selected families. The sample consists of 80 two-children families, 20 in each of four categories: early marriage and short interval to first birth, early marriage and long interval to first birth, late marriage and short interval to first birth and late marriage and long interval to first birth. The four groups are matched on socio-economic factors, and are white. Various measures of selected attitudes and personality attributes were made along with direct observations of family interaction several times during the three years of the project.

Major Findings: Age at marriage was the most potent influence upon cognitive and intellectual performance of children. First borns of late marriages scored higher on a measure of general intelligence, and both first- and second-borns of late marriages scored higher on a measure of verbal ability. First-born children of those who married at a later age had more self-esteem and were less ego-defensive. There was some congruence between parents' and children's performances on intellectual, but not on other measures.

Child-spacing was the most salient variable in relation to parents' personalities and attitudes. In general, parents with wide child-spacing possessed more psychologically desirable characteristics.

Age at marriage had more of an impact on family interaction than did child spacing. The most salient points were that First Borns in Early Marriage families showed significantly more non-compliance with maternal commands and these mothers gave approximately 50 per cent more commands than their Late Marriage counterparts.

Significance to Biomedical Research and Program of the Institute: This study is relevant to the Institute's interests in the consequences of childbearing patterns.

Proposed Course: This contract expired 1 October 1977.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title	:	A Study of Couples' Motivations for Parenthood, Decision-Making, and Fertility Regulation
Contract Number	:	NO1-HD-52807
Contractor	:	University of California, Los Angeles
Money Allocated	:	$125,209(1975);$62,925(1977);$142,463(1978):$140,000(1979)

Objectives: The aim of this project is to examine the relationship between motivations for parenthood, division of power and responsibility in the marital dyad, and fertility behaviors. Six hundred couples, with wives aged 15-34, will be interviewed 2 times over a 3 year period. Half the couples will have recently married for the first time, and the other half will have recently had their first child. In each subgroup, one member of each couple, half of the husbands and half of the wives, will be designated as primary respondent, from whom the majority of the information will be collected.

Major Findings: Preliminary results based on cross-sectional data and intra-couple analysis show that spouses who disagreed regarding number and spacing of children a) desired a larger family size, b) less frequently discussed family size and sexuality issues, and c) less frequently communicated regarding their feelings, than did spouses who agreed regarding family size. For recently married couples, actual disagreement regarding fertility was associated with full-time employment, high education and high socio-economic status of wife. Among those couples with first babies who reported that they actually disagreed at time of marriage, wives have changed more than husbands in number of children wanted. The reverse trend occurred among newly married childless couples. The birth of a first child is associated with more rather than less uncertainty regarding future fertility. Babied couples were more uncertain than Married couples regarding timing of next child and no more likely to agree on total number of children wanted. (Sixty-five percent of married couples agreed on total number of children wanted.) Comparison of actual agreement regarding family sizes (based on the number of children each spouse said s/he wanted) and stated agreement of either spous revealed only 78% of wives and 74% of husbands were accurate in their perceptions regarding agreement of the couples on current family size desires. Generally, Babied wives were more accurate in predicting spouses' desires. In cases where husbands stated they disagreed with their spouses regarding fertility, husbands thought their wives wanted more children than they actually wanted.

Significance to Biomedical Research and Program of the Institute: This study is relevant to the Institute's interest in the antecedents, determinants and correlates of fertility.

Proposed Course: This study underwent long delays in forms clearance and field work continues. It is anticipated that the study will be completed in FY 81.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : A Longitudinal Study of Intended and Actual Fertility: The 1975 National Fertility Study
Contract Number: NO1-HD-5-2819
Contractor : Princeton University
Money Allocated: $589,00 (1975); $228,945 (1976 TQ); $230,614 (1977); $215,990 (1979)

Objectives: Toward the end of 1975, some 3000 white women who were interviewed in the 1970 National Fertility Study, specifically all those married at less than 25 years of age, and not before 1951, whose first marriages are still intact, were reinterviewed, along with some 1000 white women of comparable marital status and age at marriage who married during the years 1971-75, to collect complete pregnancy histories and a record of use of the various modes of fertility regulation (contraception, abortion, and sterilization) vis-a-vis each pregnancy. Data collected consisted of both retrospective and prospective fertility intentions, attitudes toward reproduction and modes of fertility control, as well as information of explanatory value concerning such topics as experience in the labor force, attitudes toward sex roles and population problems, etc.

Major Findings: This study clearly indicates that sterilization is becoming the major method of limiting family size in the United States. It is estimated that between two-thirds and three-quarters of once-married white couples will obtain sterilizing operations within 15 years after their last wanted birth. The study also shows that the number of births intended, desired, and regarded as ideal all declined substantially between 1970 and 1975. In addition, improved control of fertility has reduced the incidence of unintended births. These factors help to explain the current low level of fertility in the United States.

Significance to Biomedical Research and Program of the Institute: The study is significant from an empirical standpoint, because no previous epoch of American fertility history has shown such dramatic change as that currently under way, and from a methodological standpoint, because this is the first attempt to use a longitudinal design with a national sample, and thus to open up the possibility of genuine causal analysis in this research area, as well as to provide a basis for the assessment of the reliability of the vast amounts of data which have been collected in the frequent, conventional cross-sectional surveys. It will contribute materially to the Center's program of understanding changes in fertility behavior of American women.

Proposed Course: This study was initiated in FY 75, and the current series of analytical studies is expected to be completed during FY 80.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : The Impact of Childbearing and Spacing Patterns on the Health of the Mother and the Development of the Child in an Urban Population
Contract Number: NO1-HD-5-2835
Contractor : Brown University
Money Allocated: $138,073 (1975)

Objectives: This study, which builds on a previous contract in Aberdeen, Scotland, focuses on the consequences of different childbearing and childrearing patterns at various ages. Data pertain to: (a) the health status of the mother for all 80,000 pregnancy events that occurred in Aberdeen, 1948-1973; (b) age, marital status and socio-economic status of the mother and father; and (c) results of mental and physical tests administered to all 12,000 children born in Aberdeen, 1950-1954, through the age-11 school examination.

Major Findings: Pregnancy histories of 3100 once-married Aberdeen women show that fertility varied inversely by social class position among non-mobile women. Upwardly mobile women tended to postpone their first pregnancy outcome and to have lower fertility than other women in the same social class of origin. Higher status women are adopting the lower class practice of starting reproduction at younger ages. Moreover, younger women are more likely than older women to have begun reproduction prior to marriage and to have concentrated their fertility in the early part of the reproductive period. Although both sterilization and termination of pregnancy are readily available, women are not adopting such measures until they have already had several births at closely spaced intervals. Pregnancy wastage is cumulative: as the number of pregnancies resulting in wastage increases, the probability of having another wastage also increases. Social class in Scotland is the major variable accounting for differences in either physical or mental development; second is birth weight for physical development, and family size for mental development. Physical development at age 5 varies directly by social class--with increase in class status there is an increase in the proportion of taller and larger children. Mental development, as measured by the 7+, 9+, and 11+ tests, similarly increases directly by social status, but at each status level the average scores decrease with each increase in family size. Low birth weight has a negative impact on both physical and mental development. Large families, higher order births, low birth weight, low socio-economic status, and very young mothers all have a sizable and detrimental impact on both mental and physical development.

Significance to Biomedical Research and Program of the Institute: The rich Aberdeen longitudinal data on the effects of childbearing and spacing patterns on the health of the mother and the development of the child are central to the Institute's concerns.

Propsoed Course: This study is scheduled to be completed in FY 80.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : An Economic Analysis of the Consequences of Child Spacing and Related Fertility Behavior
Contract Number: NO1-HD-5-2837
Contractor : The Rand Corporation
Money Allocated: $127,000 (1975)

Objectives: This economic inquiry is assessing the effects of spacing, timing, and numbers of children on children's economic well-being, parent's economic well-being, and the relationship between a wife's earnings and her period and expected fertility. Data sources include the National Longitudinal Survey and the Michigan Income Dynamics Panel.

Major Findings: When decisions concerning the quality (measured by years of schooling for the child) and quantity dimensions of children are estimated using simultaneous equation techniques, there exist strong substitution tradeoffs--e.g., when high levels of education are desired for children, the number of children is low. The opportuntiy cost of a woman's time plays a central role in these tradeoffs. The proportion of boys reduces desired family size, as there is higher desired "quality" of boy children as measured by years of schooling completed. In the interactive quality-quantity model, higher desired quality increases the cost of numbers of children; and any undersired fertility induces a substitution away from quality.

The number of children a woman rears reduces her lifetime work experience--(on the average) by 2.5 years for the first child, two more years for the second child, and another year for the third. Longer birth intervals (holding constant number and timing of children) are associated with larger reductions in lifetime work. The number of children borne has a greater effect on lifetime work experience than does the age at which a woman has her first child. The market wage rate of return increases 4 percent for each additional year of work experience. The average life-cycle wage of a childless woman is 13 percent higher than that of a woman who has three children spaced three years apart beginning at age 22; the maximum difference in wage rates (22 percent) occurs at age 32. As for the effect of children on a family's accumulated net worth, family size has a negligible impact on total net worth or its growth, but substantially alters the composition of net worth. Larger families increase the fraction of a family's wealth in durable assets and reduce financial assets proportionately.

Significance to Biomedical Research and Program of the Institute: The analysis of the economic consequences of fertility behavior is central to the program of the Center.

Proposed Course: This study was completed in FY 78.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title	:	Analyses of Relationships between Fertility, Marriage Dissolution, and Remarriage
Contract Number	:	NO1-HD-52838
Contractor	:	Research Triangle Institute
Money Allocated	:	$154,130 (1975); $79,529 (1976); $14,634 (1978)

Objectives: This project uses existing data--the 1967 Survey of Economic Opportunity, the 1971 Marital and Fertility History File, and the National Fertility Surveys of 1965, 1970 and 1975--to analyze childspacing in relation to changes in marital status of mothers of not more than four children. A classification system combines childspacing and marital history data into indices with detailed socio-economic and attitudinal variables. This study builds on the work of others in the area of marital disruption and fertility and offers new insights into the reciprocal effects of fertility and marital dissolution.

Major Findings: This study of the relationship between fertility, marriage, divorce, and remarriage shows that divorce is more likely for women who had given birth before marriage, for those who were less than 18 years old when first married, and for those who conceived before marriage but had the child after marriage. Among those women who divorced and remarried, childbearing in the second marriage did not compensate for the smaller number of children resulting from divorce.

This study supports the hypothesis that childlessness facilitates divorce, while the presence of children deters divorce -- in fact, each additional child reduces the probability of divorce and delays divorce. The study also shows that the presence of young children does not reduce the divorced mother's chance of remarriage.

Significance to Biomedical Research and Program of the Institute: This project offers insights into two forms of behavior which have health and social consequences both for the individual and society--fertility and marital dissolution, a relationship that is complex and most likely reciprocal. This study provides the basis upon which other indepth studies can be built.

Proposed Course: This project was completed in FY 78.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title	:	Research on the Consequences of Childbearing and Childspacing Patterns for Parents
Contract Number	:	NO1-HD-52840
Contractor	:	Battelle Memorial Institute
Money Allocated	:	$94,860 (1975); $97,885 (1976 TQ)

Objectives: This project focuses on the effects of age at first marriage, age at first birth and the number and timing of births on parental and marital satisfaction; marital careers; occupational and economic activities of parents, geographical mobility, and non-work activities. The effects of childbearing and childspacing on these outcomes were examined for both women and men. This analysis was based on data collected from a study by Coleman of high school students and a 15-year follow-up of these same respondents.

Major Findings: The older the age at first marriage, and therefore the age at first birth, the more satisfied one was as a parent. The more children one had, on the other hand, the less satisfied one was. The presence of children had a negative effect on marital satisfaction and was more pronounced the younger the age of the youngest child. Premarital pregnancy had a negative effect on parental and marital satisfaction of women but no effect on men. Generally, premarital pregnancy had little effect on males, but it was found to have a small effect on their use of leisure time. Men involved in a premarital pregnancy belonged to fewer voluntary organizations and spent more time watching television. Childbearing patterns were found to be related to women's occupational behavior but not to men's. Number of children had a strong negative effect on women's participation in the labor force and also on gains in the occupational prestige and earnings of women in the labor force.

Childspacing, as measured by the length of the average birth interval, affected few measures of the behavior and attitudes of parents. However, one area was in the importance attached to the chance to have and rear children as an aspect of marriage. Mothers and fathers whose children were spaced closer together ranked the chance to have and rear children as a more important aspect of their marriages than those who spaced their children farther apart. The only other apparent effect of childspacing was observed in relation to migration. Specifically, rapid childbearing after marriage was found to have a small inhibitory effect on migration, which appeared to arise because those who bore their children sooner after marriage had school-age children sooner, and the presence of school-age children had an inhibitory effect on migration.

Significance to Biomedical Research and Program of the Institute: This study will be valuable in the insights it will gather to the interrelationship of fertility behavior and social and psychological well-being. Fertility behavior has both biomedical and social consequences and this research will elaborate on how biological phenomena result in social and psychological outcomes.

Proposed Course: This project was initiated in FY 75 and was completed in FY 79.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : A Study of Low Fertility Cohorts in the United States
Contract Number: NO1-HD-3-2799; NO1-HD-6-2818
Contractor : Gerogetown University
Money Allocated: $99,911 (1973); $16,140 (1974); $22,913 (1975); $340,153 (1976); $424,410 (1977); $167,227 (1978)

Objectives: This study seeks to gain a deeper understanding of the low fertility of the 1920s and 1930s in the United States, by studying the cohorts of women born during 1901-1910. These cohorts achieved the lowest completed fertility recorded in the demographic history of the United States. After careful instrument development and pretesting a field survey will be undertaken to interview 1000 women and their husbands. The questionnaire will include items on background characteristics, pregnancy and contraceptive history, attitudes toward family size as well as measures of the influence of the Depression on their lives.

Major Findings: A majority of the women (70 percent) reported that they or their husbands had used a method of contraception. The most commonly used method was the condom, with douche and withdrawal following in that order. Among the women who reported not using a method to prevent pregnancy, 57 percent reported douching for hygienic reasons. In addition, one-fourth of the women reported having had an operation that caused them to be sterile before age 50.

Results of the study show that the couples most severely affected by the Depression had more children than those less affected -- partly because those most affected were a select high-fertility group. It is hoped that the causal direction of this association will be revealed by further analysis.

Significance to Biomedical Research and Program of the Institute: As part of the Institute's interest in understanding the determinants and consequences of fertility, this project will fill a major gap in our understanding of how and why women restricted their fertility. The women under study had the lowest total fertility rates ever recorded in the U.S. and yet they accomplished this prior to the introduction of most "modern" contraceptives. The motivations of these women and the means they used to implement these motivations will bear on our understanding of the general issue of birth control and motivations for parenthood.

Proposed Course: Initial analysis of the data from this survey will be completed during FY 80.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Development of Technology and Training Program for Assessing Unit Record Data Files
Contract Number : NO1-HD-22707; NO1-HD-62828
Contractor : National Data Use and Access Laboratories, Inc.
Money Allocated : $235,403 (1972); $252,296 (1974); $214,440 (1976); $110,571 (1977); $43,137 (1978)

Objectives: The purpose of this contract is to develop techniques to facilitate the wider use of public use data files, such as the Public Use Sample of the Bureau of the Census. The project emphasis is on the handling of very large files by the writing of COBOL and FORTRAN programs which will allow available computer packages (SPSS) to be used with the Census data and to enable researchers to assess large complex files with a minimum of programming and maximum speed. The project also provides for the preparation of users' manuals and provision of classroom instruction as well as maintenance of the package for those who acquire them.

Major Findings: During the course of this contract DUALabs has created two basic computer software systems--PUSH and CENTS-AID. PUSH enables researchers to take the very large, hierarchical file of the Public Use Sample File of the Census Bureau and analyze it with SPSS,(Statistical Package for Social Sciences) a widely available and easily used analysis package.

CENTS-AID provides rapid cross-tabulation of very large files, including complex hierarchical files. A minimum of programming is required and this, in conjunction with its speed of running, results in very inexpensive analysis of large files. CENTS-AID II is a cross-tabulation program which provides some statistics (mean, median, standard deviation, percentage distribution). Version 3.0 is now able to create computer readable files for use in other programs and generate sample report pages. It allows greater flexibility in the format of the data to be processed and provides a higher level of accuracy within the tally cells. A correlational subroutine has been included with options which allow for flexibility in both the data input and output formats. Documentation has also been made available.

Significance to Biomedical Research and Program of the Institute: The U.S. Census is one example of the important public data sources available for demographers and other social scientists. The value of these very expensive data sets has been limited by the cost and complexity of their analysis. The programs developed under this contract serve to open up many public use files for analysis and greatly enhance their value. Benchmark comparisons demonstrate that CENTS-AID can reduce tabulation costs up to 80%.

Proposed Course: This project is scheduled to terminate in FY 79.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : The Consequences of Early Childbearing: An Analysis of Selected Parental Outcomes Using Two Longitudinal Data Sets
Contract Number: NO1-HD-6-2829
Contractor : The Urban Institute
Money Allocated: $116,536 (1976); $122,190 (1977)

Objectives: To assess the effects of early childbearing on educational attainment, completed family size, socio-economic status and income, marital disruption, probability of being on welfare, independent of the impact of background factors such as parental status, race, and year of birth. Two complimentary longitudinal data sets are being used for the analysis of these issues. The Michigan Panel Study of Income Dynamics and the young female sample from the National Longitudinal Survey.

Major Findings: Analyses indicate that an early birth reduces the amount of schooling a young woman is able to complete even with controls for family background, motivation, and age at marriage. This result occurs particularly among white and high school age mothers. Early childbearers do not appear to catch up over time; indeed they appear to fall further behind as their childless peers continue in school. Teenage mothers also have substantially larger families, even when other factors are taken into account.

While a youthful age at first marriage was found to predict divorce and separation, early childbearing was not found associated with a higher probability of marital disruption, except indirectly by precipitating teenage marriages. Age at first birth was found to have no direct effect on work experience, occupational status, wages, annual earnings, welfare recipiency or poverty. However, early childbearing appears to have an indirect impact through effects on education, marriage and family size. To explore the implications of these indirect effects for the probability of poverty, causal models were developed and estimated. Results indicate that the impact of an early birth is complicated and differs among different population sub-groups. Early childbearing was associated with greater poverty in both samples. Each year of delay reduces the probability of poverty by 1.6 percentage points for NLS women aged 27 and by 1.3 percentage points among PSID women.

Significance to Biomedical Research and Program of the Institute: The issue of adolescent childbearing is of concern both in the social and medical arenas. Besides having effects on both indicators of social well-being (education, socio-economic status) and medical well-being (incidence of low-birth-weight babies, complications of pregnancies) these two phenomena most likely interact.

Proposed Course: This project was completed in FY 79.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Alienation and Fertility Control in the Early Years of Marriage
Contract Number : NO1-HD-6-2833
Contractor : Bowling Green State University
Money Allocated : $142,361 (1976); $125,117 (1977); $111,309 (1978)

Objectives: This proposal involves testing of the relationship between alienation and fertility with respect to a broad range of dependent variables, including family size preferences, early contraceptive and pregnancy decision-making, contraceptive use, premarital and unplanned pregnancies, and abortions. The research will focus on the early years of childbearing. Data will be collected by means of self-administered questionnaires from a group of 600 once-married couples, classified according to wife's age at marriage and marital duration. Methodologically, in addition to development of new measures of alienation, reversal of the alienation concepts will result in focus on positive measures of social integration. This work continues and expands on previous work by the principal investigators.

Significance to Biomedical Research and Program of the Institute: This research is relevant to the Institute's interests in the antecedents and determinants of fertility.

Proposed Course: Forms clearance was received in FY 78 so field work is now under way and this study is scheduled for completion in FY 80.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Fertility Values and Family Growth
Contract Number: NO1-HD-6-2834
Contractor : Temple University
Money Allocated: $180,753 (1976); $30,049 (1978)

Objectives: The goal of this project is to study the developmental effects of actual fertility on fertility values within religious and SES subgroups. This project is the outgrowth of a successful methodological study (contract number NO1-HD-12241) regarding the conceptualization and measurement of fertility values. That study indicated that the experience of bearing children was related to people's perceptions of a range of values related to fertility and family-size desires. In that study, however, because longitudinal data were not available, it could not be determined whether people with three children had different values from those with no children because of the presence of children. The study proposed here will establish more clearly: (a) the effects of parity increase on the relationships of fertility values to desired family size; (b) the fertility value relationships that are associated with Catholic/Protestant affiliation, social class and those that are independent of these two factors; and (c) the parental experiences associated with an increase in parity.

Significance to Biomedical Research and Program of the Institute: This project is based on a successful exploratory study regarding fertility values. Through the careful identification of the criteria by which people evaluate various family sizes and assess their costs and rewards, this research will enhance our understanding of the process of family formation. The research will also improve our understanding of the consequences of family size by elaborating how fertility influences future decisions about having children. The bearing of children--family formation--is a process in which decisions are continually made and revised but it is this aspect that is most difficult to research. To gain insight into this process would be a significant advance in the field of population research.

Proposed Course: The first stage of this project is expected to be completed in FY 80.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : The Social and Demographic Consequences of Teenage Childbearing for Urban Women
Contract Number : NO1-HD-62836
Contractor : The University of Maryland
Money Allocated : $101,246 (1976); $9,912 (1978)

Objectives: The purpose is to investigate the impact of age at motherhood on the role aspirations and behavior of women, specifically assessing the consequences of having the first birth during the teenage years versus postponing it until a woman is in her twenties. Data for the analysis are from a longitudinal study of 408 new mothers in New York City.

Major Findings: At the first interview (when the first child was ½ to 2½ years old) one-half of the teenage mothers said they wished the child had been born later or never. Three years later 78% of the teenage mothers said that looking back they would choose a later age for their first birth. Despite this, teenage mothers were more likely to have a second child within two years after their first than were mothers in their twenties.

Over one-fourth of the unmarried mothers had married within the 45 months covered by the study, about 2/3 of them to the child's father. However, 37% of these women became separated or divorced within this short period. Two-fifths of the mothers had a second child within 45 months; 56% of these second births were also out-of-wedlock. Over half of the young mothers had been employed, about half returned to school and 30% had returned to school and were employed (not necessarily simultaneously). Not surprisingly, over 86% of the teenaged mothers were at some time since the birth in a household receiving public assistance. Less than one-fourth of the fathers of babies born out of wedlock were in contact with the child's mother on a weekly basis at the time of the second interview, and contact diminished over the years. Maintaining social contact seems linked with financial support, rather than being a substitute for it. None of the unmarried mothers received child support in all three years covered. The young mothers' parents, especially her mother, provide housing, childcare, and in over 2/3 of the cases direct financial aid. Seventy-seven percent of those working or going to school relied on relatives to care for their children. Friends also helped the mother with babysitting.

Birth control practice changed dramatically after the birth of the first child. The majority of these out-of-wedlock mothers (57%) had never used contraception before becoming a mother. By the time of the third interview, only 13% of these women had never used contraception. There was also a shift to more effective methods of contraception and the use of abortion.

Significance to Program: The consequences of adolescent childbearing are of concern in the United States both in terms of social and medical consequences. This project addresses how early childbearing influences the future lives of women, using an excellent longitudinal data set.

Proposed Course: This project was completed in FY 79.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Sequelae to Teenage Pregnancy
Contract Number : NO1-HD-62858
Contractor : Princeton University
Money Allocated : $74,891 (1976); $83,542 (1977)

Objectives: The purpose is to assess the consequences of adolescent childbearing on marital stability, later fertility, education, labor force participation, income and poverty status, attitudes of various kinds and selective consequences to the children. These topics will be addressed through the National Fertility Surveys of 1965, 1970 and 1975 as well as the 1973 National Survey of Family Growth.

Significance to Biomedical Research and Program of the Institute: The consequences of adolescent childbearing are of particular interest in the United States where one birth out of five occurs to a teenager. These effects may be felt in both the medical and social spheres of life.

Major Findings: This research has demonstrated that marital dissolution rates decrease as the age at first birth increases. Younger ages at first birth are strongly associated with marriage within a relatively short period of time after the first birth, higher proportions of unwanted and illegitimate children, and a faster pace of subsequent childbearing and completed fertility. These conclusions hold within racial, educational, and religious subgroups. However, some racial differences have been found in the area of marriage. A nonmarital conception of a live birth is far more likely to lead to a marriage to legitimize the birth among whites than among blacks. Also, following marital disruption, whites are more likely to remarry than blacks. Racial differences in marital disruption diminish with regard to second marriages dissolution. Women who had a first birth prior to or in their first marriage are more likely to have their second marriage dissolve if the first child was born before the mother's twentieth birthday. This analysis suggests, however, that 18-19 year olds, who indeed experience somewhat lower fertility and fewer unwanted births than younger women, are still a higher risk group than women whose first birth occurs after 20. Analysis of patterns of contraceptive use and effectiveness according to age at first birth has shown little difference either in current use or in current effectiveness of contraceptive use when women who became mothers as adolescents are compared to those who postponed childbearing beyond age 20. Analysis of family structure indicates that the proportion of children of any given age between zero and 18 who have ever lived in a single parent household is strongly and inversely related to the age of the mother at first birth, with the proportions being lower for whites than for blacks. Also, the younger the mother at first birth, the higher the proportion of children whose household type has changed and the lower the proportion living continuously since birth with both parents.

Proposed Course: This project was completed in Fiscal Year 1979.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Research on the Consequences of Adolescent Pregnancy and Childbearing
Contract Number : NO1-HD-6-2859
Contractor : University of North Carolina
Money Allocated : $61,866 (1976); $94,332 (1977)

Objectives: This study assesses the long-term, social and demographic consequences of teenage childbearing through the use of the 1971-1975 Current Population Surveys, the 1970 National Fertility Survey, and the 1973 National Survey of Family Growth. The CPS and the NFS offer different kinds of data and sample sizes and provide complementary data sources. The effects of adolescent childbearing on subsequent fertility and marital history are being studied by descriptive and life table analyses of the timing and sequence of birth and marital events of age-at-first-birth cohorts. The effects of adolescent childbearing on subsequent socioeconomic achievement are being explored initially by comparing the means and variances of a number of socioeconomic statuses for age-at-first-birth cohorts by age at interview. Finally, reasons for socioeconomic differences between adolescent childbearers and post-adolescent childbearers are being examined by estimating past models of indices of family economic welfare and other variables.

Major Findings: Data suggest that among all cohorts (women bearing their first child in 1930-1945, 1946-1960, and subsequent to 1960), adolescent mothers had higher fertility, and shorter spacing of subsequent births. The majority of mothers with only one child at the time of survey are expected to have an average of one more child in their lifetime regardless of age at first birth. Among mothers with two births, however, a higher percentage of adolescent mothers, as opposed to post-adolescent mothers, are expected to have one more child. This research has also documented a number of other important findings indicating that childbearing at unusually young ages results in a number of undesirable outcomes for both whites and blacks, e.g., less education, lower occupational status, a higher prevalence of poverty, lower family economic welfare, and higher rates of marital dissolution.

Significance to Biomedical Research and Program of the Institute: The consequences of adolescent childbearing are of particular concern in the United States where one birth out of five is to a teenager. These consequences may be social and/or medical and affect the mother, the child, the father, extended family and society. This project fits into a broad spectrum of research designed to measure the consequences of adolescent childbearing and document the processes by which such consequences result.

Proposed Course: This project, begun in FY 77, will be completed in FY 79.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Effects of Adolescent Pregnancy on Mother-Infant Relationships: A Transactional Model
Contract Number : NO1-HD-6-2860
Contractor : George Peabody College for Teachers
Money Allocated : $138,264 (1977); $61,569 (1978)

Objectives: To analyze existing data in three studies evaluating the psychological and behavioral consequences (among socio-economically disadvantaged patients in Nashville General Hospital) related to early childbearing among pregnant adolescents in comparison with pregnant women above age 20.

Major Findings: This sample of newborns indicates significant relationships of age at menarche with maternal age in support of current theories of increased fecundity among younger women and implies that the younger pregnant adolescent may be biologically prepared for reproduction at an earlier age and is not necessarily more sexually active than her nonparent age mates. In conjunction with the findings of others, this suggests that menarchal age may be of more consequence than chronological age in adolescent fertility. In addition, this research supports the hypothesis that younger, adolescent women pregnant for the first time experience more stress than do older women and women who have had several pregnancies. It also shows a significant relationship between the effects of anesthesia given to the mother predelivery and the behavior of the newborn, with a high dose given shortly before delivery resulting in the greatest behavioral decrements as measured by the Brazelton Behavioral Assessment Scale. Other studies have shown type and timing of medication to be related to parity and length of labor. Primiparous mothers are more likely to have more and stronger drugs administered. As the adolescent mother is more likely to be primiparous, the effect of drugs on the children of adolescents, operating through parity, may be considerable. Analyses also have shown that older mothers both talk to and look at their babies more often than do younger mothers. Increased talking and looking time were associated with higher scores on measures of motor and mental skills, with more positive maternal attitudes, such as closeness to the child, competence in meeting the baby's needs and encouragement of reciprocity, and with more positive perception of the baby's temperment. These findings suggest that interaction patterns play an important role in the social development of the child.

Significance to Biomedical Research and Program of the Institute: This project is relevant to RFP 76-11 on "Research on the Consequences of Adolescent Pregnancy and Childbearing" and provideinformation on mothers at risk of mistreating their children and other information on the effects of prenatal training programs of use to the policymaker.

Proposed Course: This three-year contract is scheduled to be completed in FY 79.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title	:	The Intellectual and Behavioral Status of Children Born to Adolescent Mothers
Contract Number	:	NO1-HD-72805
Contractor	:	The Alan Guttmacher Institute
Money Allocated	:	$46,704 (1976); $45,720 (1978); $40,277 (1979)

Objectives: This study makes use of Cycles II (1963-1965) and III (1966-1970) of the Health Examination Survey (ages 6-11 and 12-17) and the Collaborative Perinatal Project data on children 7 years old (1959-1973). The focus will be on differences between children of youthful mothers and other children of the same age with respect to intelligence, achievement, and personality adjustment as measured by tests and observation of behavior. In addition, the development of children born of adolescent mothers will be compared with that of their siblings who were born later, after their mother had passed adolescence. The analysis will be shown by single years of age of the mother, whereas earlier findings from the source material have been shown for women under 20 years old as a group. The value of this study lies in its attempt to establish, or at least clarify, causal relationships between being born of a teenage mother and the performance variables included.

Significance to Biomedical Research and Program of the Institute: This research is relevant to the Institute's interest in the correlates and consequences of fertility.

Major Findings: Analyses of the three data sets have shown consistently that maternal age has a very small direct effect on the child's IQ. However, the indirect effect of early childbearing operating through social and demographic variables, such as race, mother's education and income, is clear. Attempts have been made to understand the association between mother's age and negative consequences to the child. Effects of maternal age on the child's social and emotional development were limited. However, results showed that in the area of parent's expectations and aspirations for the child, older mothers had greater hopes for their children's futures than younger mothers and also gave their children more responsibilibies. In addition, children of younger mothers were perceived as being more difficult to raise and less attentive, as well as having more adjustment problems. A "parenting" factor, while not related to maternal age, was identified as one way in which demographic factors may get translated into IQ differences in children. An interrelationship was found between birthweight, IQ, and maternal age, but the small independent effect of maternal age on IQ remained after birthweight was controlled. Thus, birthweight is not the mechanism through which the maternal age effect is manifested.

Proposed Course: This study began in November 1976 and is expected to be completed in November 1979.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Economic, Social and Psychological Consequences of Teenage Childbearing
Contract Number : NO1-HD-7-2806
Contractor : Institute for the Continuous Study of Man
Money Allocated : $64,607 (1977); $77,674 (1978)

Objectives: This is a study of the consequences of adolescent childbearing in black and white populations of lower socioeconomic status. The data is a sample from the Philadelphia National Collaborative Study (10,000 pregnancies of which about 25% are under 20 years of age). A large number of economic, social and psychological consequences are to be analyzed with reference to antecedent variables. Data on child development to age seven are available, including behavior in the home and at school, psychological problems measured behaviorally and interaction with parents.

Major Findings: Analyses show that blacks giving birth under 18 were disadvantaged in a number of areas compared to older women: at the time they gave birth, they were more likely to have lower incomes; the fathers of their babies were less likely to be present or to be married to them; they had completed fewer years of school and they were less likely to have prior work experience. Disadvantages remained on a 7-year follow-up. Though there were no differences in reported incomes, younger women were more likely to receive public assistance and were less likely to be living with husbands. They remained less well-educated.

Findings concerning the effects of maternal age on the child suggest a tendency for children born to mothers under 18 years of age to have slightly lower IQ test scores than children of older mothers. This tendency was more marked for black children than for white children and for boys than for girls. Measures of the child's social and emotional behavior showed no significant effect of maternal age at 4 years of age. However, measures taken at 7 years of age indicated that children born to mothers under 18 were more prone to behaviors reflecting a lack of impulse control, over-activity, and hostility or resistiveness. Again, the effects were in evidence primarily in the black sample and they were especially pronounced for boys.

Significance to Biomedical Research and Program of the Institute: This research is relevant to the Institute's interests in the correlates and consequences of fertility.

Proposed Course: This study started in July of 1977 and is expected to take about 26 months to complete.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Consequences of Late Adolescent Childbearing in the Early 1970s
Contract Number: NO1-HD-7-2819
Contractor : The Rand Corporation
Money Allocated: $120,700 (1977); $131,130 (1978); $46,600 (1979)

Objectives: This research examines the near-term consequences of late adolescent childbearing in five major areas of the young parents' lives: (1) dimensions of personality (self-esteem, locus of control, and adult role orientation); (2) future expectations and aspirations about work, education, and family growth; (3) women's perceptions and orientations about homemaking and work; (4) young parents' economic well-being; and (5) their economic dependency on their parents and society. Data are from the National Longitudinal Study of a nationally representative random sample of about 22,000 seniors of the class of 1972 from 1200 high schools. Follow-up waves were conducted in the Fall of 1974 and 1976. Analyses focus on chains of causation through which adolescent childbearing predisposes specific consequences for the parents.

Major Findings: Late adolescent parents differ considerably from nonparents on many of the outcome measures. The differences shrink, however, when one allows for differences in background characteristics related to the outcome variables and shrink further for married parents compared with married nonparents. Among married women, early parenthood is associated with differences in career aspirations. Mothers tend to score lower on work orientation measures, select homemaking as the most likely career at age 30, and to be less satisfied with their career progress. On average, adolescent parents expect to have slightly larger families than do nonparents, and an association was found between timing of childbirth and welfare dependency.

Significance to Biomedical Research and Program of the Institute: Adolescent childbearing is of particular concern in the U.S. where one birth in five is to a teenager. It has both social and medical sequelae which in all probability interact to influence the future life course of those involved. This very important area of research has lacked definitive analyses of these consequences. This project is designed to help fill this gap.

Proposed Course: This project is scheduled for completion in FY 80.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Long-term Consequences for Children of Adolescent Childbearers: An Analysis of Project TALENT Data
Contract Number: NO1-HD-7-2820
Contractor ; American Institutes for Research
Money Allocated: $72,671 (1977)

Objectives: Project TALENT longitudinal data from a national sample of the 1960 high school population were used to investigate whether children born to adolescent parents differ from their peers on a variety of long-term outcomes--cognitive and social-psychological variables measured during adolescence; and educational, occupational, marital and fertility outcomes taken at about age 30.

Major Findings: A previous analysis of Project TALENT data (Card, J. J., Consequences of Adolescent Childbearing for the Young Parent's Future Personal and Professional Life. Palo Alto, Calif.: Am. Institues for Research, 1977) found that teenage childbearing had enormous impact on the future educational, occupational, marital, and family lives of young parents, especially young mothers. The present study has found much smaller consequences for the future lives of the children involved. The main consequences for these children center around: (1) a greater probability of living in a non-intact (single-, step-, or no-parent) home while in high school; (2) lower academic aptitude as a teenager, brought about partially by this different family environment; and (3) a slightly increased probability of repeating the parents' pattern of early marriage, early parenthood, and greater fertility. The unique impact of adolescent parentage on these outcome variables, though statistically significant, was small, being surpassed by other background characteristics such as race and socioeconomic status. No significant differences were found in the magnitude of the cognitive consequences of adolescent parentage for various subgroups of children (males vs. females, blacks vs. whites, individuals from small vs. large communities, poor vs. rich, or first- vs. later-born children).

Significance to Biomedical Research and Program of the Institute: The study of long-term consequences for children born to adolescent parents is of value to the Institute because the implications for adjustment and attainment of the children over a large portion of their lives can be assessed.

Proposed Course: This project was completed in FY 79.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Consequences of Teenage Motherhood for Mother, Child and Family in a Black Urban Community
Contract Number: NO1-HD-7-2821
Contractor : University of Chicago
Money Allocated: $174,966 (1977); $247,963 (1978)

Objectives: The proposed research is studying the sociological, psychological and psychiatric consequences of teenage childbearing for the mother and for the child born to them. The project will analyze data collected for the Social Psychiatry Study Center's longitudinal, community-wide study of children and their families in a black, low-income Chicago community. The study sample consists of 1242 children who entered the first grade in 1966, over 200 of whom were born to teenage mothers and over 500 of whose mothers began childbearing as teenagers. The data include: periodic ratings and assessments of the childrens' social role functions and psychological well-being, IQ, grades and achievement measures collected over a 10-year period, personal interviews with the mothers in 1967 and 1975 providing information on demographic, social interactonal, and psychological variables for the mother and the family; and baseline and outcome information for those who participated in a project-related treatment program.

Major Findings: Children of mothers who began childbearing at 17 or younger are less likely to be rated as adapting to school by first-grade teachers (time 1) than children of older mothers, and, particularly for boys, failure to adapt to first grade is strongly related to more intense psychiatric symptoms 10 years later. Mother's age at start of childbearing is related to her psychological well-being when the study child was in first grade (time 1), young teenage mothers being least likely to report feeling very good and most likely to report feeling very bad at this time. All mothers' psychological well-being, if low at time 1, is at risk of remaining low ten years later (time 2). Teenage mothers are more likely than older mothers to be the only adult in their household (mother alone) at time 1, and least likely to be in mother/father households. All mothers in mother-alone household at time 1, regardless of age of start of childbearing, are at high risk to be in mother-alone families at time 2, but teenage mothers in mother/father households at time 1 are less likely than older mothers to remain in mother/father families at time 2.

Significance to Biomedical Research and Program of the Institute: Evaluation of the consequences of teenage childbearing has special relevance to one of the major emphases of the research program of the Center for Population Research: the consequences of childbearing patterns to mothers, children, and society. The problem of teenage childbearing is especially significant because of the increasing rates of childbearing among teenagers, especially the younger teenagers. The study will provide essential information regarding health, welfare and social adjustment problems for both mothers and children of significance to a variety of concerns of the P.H.S. and the D.H.E.W.

Proposed Course: This contract, initiated in FY 77, is expected to be completed in FY 80.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Early Childbearing and Kinship Relations
Contract Number : NO1-HD-7-2822
Contractor : Center for Research on the Acts of Man
Money Allocated : $60,150 (1977); $5,758 (1979)

Objectives: This research is investigating how the pregnancy and childbearing experiences of teenagers interact with the life situation of other family members. The specific aims of the study are: (1) to describe the availability of kin services to the mother and identify and conditions that affect such services; and (2) to study the impact that providing such services has on the kin members themselves and on kin relationships. Data sets available are longitudinal survey data including non-pregnant teenagers, teenage mothers and older mothers.

Major Findings: Most of the adolescents in the sample studied were highly dependent on the family, especially in the first several years after their child was born. Some 70% of the sample were living with one or both parents the year after their child was born, and, at the five year follow-up, more than a third were still residing with their parents. Factors influencing how much aid was provided by the families were: (1) demand factors such as the teenage mothers' desire to complete school and their emotional reliance on their parents and (2) supply factors such as the family's economic resources, the teenager's additional childbearing, and the available space in the household. The young mothers who did receive family support were more likely to complete their schooling and to become economically independent. An investigation of the long-term consequences of teenage parenthood for family members indicated that the early childbearers' nuclear family members did not experience disadvantages in their own socioeconomic and family careers as a result of the teenage pregnancy. It was also found that women who had their first child by age 17 typically had fewer kin outside their households on whom they could rely for help. 15 cases studies of teenage mothers and their families provided a microscopic assessment of their immediate adjustments. Families must decide whether or not to incorporate the child, the father, and his family; they must develop an effective division of childrearing labor and must resolve disputes; and they must reallocate rights, duties, prestige and other rewards as the statuses of family members change. The most salient finding to emerge from these case studies is that a teenage birth confers both burdens and benefits on a family, although the balance varies from one family to another.

Significance to Biomedical Research and Program of the Institute: This study will contribute to the understanding of how teenage motherhood affects the relatives of the teenage mothers and the extent to which the kinship system is relied upon for financial support and childcare. The findings will thus have important implications for the study of childbearing patterns and for HEW programs.

Proposed Course: This study is expected to be completed in FY 79.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Relations Between Teenage Pregnancy and Neonatal Behavior
Contract Number : NO1-HD-7-2823
Contractor : Children's Hospital Medical Center, Boston
Money Allocated : $11,480 (1977)

Objectives: This study investigates the extent to which adolescent pregnancy is related to behavioral deficits in the newborn, relates several prenatal and obstetric factors to behavioral outcomes in children of young mothers, and compares the behavioral characteristics of these neonates across two cultures. Data were analyzed on 150 infants from Puerto Rico and 150 from Florida who were administered the Brazelton Neonatal Behavioral Assessment Scale on the infant's second day of life. In addition, to the Brazelton, complete records of medical history of mother and baby were available for each infant. These included a variety of physical measures taken on the infant. The mothers in the Florida group ranged in age from 14 to 31 years of age with 32 mothers aged 17 or below. The mothers in the Puerto Rican group ranged from 13 to 32 years of age with 30 mothers below the age of 18.

Major Findings: Analysis of obstetric risk scores showed that the infants of teenage Mainland mothers had 1! to 2 more obstetric complications than the infants of older Mainland mothers and than the infants of teenage and older Puerto Rican mothers. This appeared to be primarily due to the single marital status of the teenage Mainland mothers. Analysis of the Brazelton scale scores showed that when teenage and older mothers were matched for obstetric history and perinatal risk factors and further divided into high and low obstetric risk groups, infants of teenage mothers show comparable scores to infants of older mothers. Neonatal behavior is affected by obstetrical risk and by the cultural membership of the infant. The findings from this study suggest that, given prenatal care, the infant of the teenage mother is at no greater behavioral risk than the infant of the older mother. Infant behavior is affected by obstetrical complications and perinatal risk factors, but these effects are independent of the age of the mother Mother's age is probably best thought of as a marker variable for increased obstetrical complications, primarily in the absence of prenatal care. Cross-cultural differences suggest that the cultural meaning of teenage pregnancy is important for understanding the sociocultural stresses that the pregnant teenager is likely to face. These stresses may affect the obtaining of proper prenatal care and thus affect her pregnancy and the outcome of the infant. They will also affect the environment in which the infant and mother must negotiate as individuals and as a dyad.

Significance to Biomedical Research and Program of the Institute: This research is relevant to the Institute's interests in the correlates and consequences of fertility.

Proposed Course: This contract expired September 1978.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : A Continuing Study of Childlessness and One-Child Families
Contract Number: NO1-HD-8-2801
Contractor : University of Southern California
Money Allocated: $67,653 (1978)

Objectives: This study proposes to complete work already begun at the University of California by the P.I. dealing with comparative levels and trends in childlessness and one-child families in the United States and around the world. The actual prevalence of childlessness and one-child fertility will be analyzed historically and comparatively. Such analyses will be used to test current theories and formulate new theories. Variations in childlessness and having one child will be examined using various demographic and social characteristics such as age, age at marriage, level of educational achievement, labor force participation of the women, occupation, rural or urban residence, ethnic identity, race, religion and income. A catalog of statistical information on childlessness and one-child fertility found around the world will be completed. Such a catalog, the first to be made anywhere, will be of use to other researchers as well as to the investigator in this study. With the reported and estimated facts available on an international basis, it is proposed to establish the range of international and historical variation in the level of ultimate 0 and 1 parity, and to discover the kinds of societies and economies that give rise to levels close to the lower and upper limits.

Major Findings: On the basis of fertility data between 1960 and 1974, it was found that the proportion of one-child families in the United States is increasing. The proportion of childless women has always exceeded the proportion having only one child, but the difference has declined over time and the change appears to have come about more through a reduction of childlessness than from a rise in the proportion of women with only one child. The study has also found that the proportion childless tends to double with every increase of five years in age at first marriage.

International comparisons show that the United States is high in both the proportion childless and the proportion with one child, controlling for age at first marriage. International comparisons also revealed distinctive differences among countries.

Significance to Biomedical Research and Program of the Institute: This study is relevant to the program of research in the behavioral sciences because it deals with one extreme of the fertility distribution curve, and can shed light on factors affecting motivation for fertility limitation. It is particularly timely in view of current social emphases upon fertility limitation and advocacy by some groups of childlessness and one-child families. The study of factors affecting such behavior provide complementary data to the behavioral science program to study consequences of family size.

Proposed Course: This contract, which was begun in FY 78 will be completed in FY 79.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Survey Research on Childlessness & the One-Child Family
Contract Number : NO1-HD-8-2802
Contractor : University of California, Los Angeles
Money Allocated ; $55,602 (1978)

Objectives: Through the use of survey techniques, this project will continue the effort initiated under a previous contract to study motivation for below-parity reproductive behavior. Using a data bank of Gallup surveys dating back to the 1940s, plus special survey questions on childlessness and the one-child family generated in February 1977 and to be commissioned in the course of this proposed contract, socio-demographic and attitudinal characteristics of persons favoring low fertility as compared with persons desiring larger families will be analyzed. The analysis will include six European surveys from the late 1960s containing data on family size preferences. This research also seeks to measure the general climate of opinion concerning childlessness and one-child families. A final objective of this research is the development and improvement of a scale to measure attitudes toward childlessness.

Major Findings: This study strongly emphasizes the unpopularity of childlessness in the United States, despite the general recognition of some of the disadvantages of life with children. Approximately 60 percent or more of respondents questioned in the 1977 survey disagreed that one submerges one's individuality when one becomes a parent, that childless married couples are more intimate, that they have more varied lives, or that a woman would be ill-advised to sacrifice job chances for maternity.

One of the more important findings is the concern for children as social investments - that is, as offsetting loneliness in old age, making a person feel more adult, and providing a goal in life. There seems to be very little general support for the values of the non-parenthood movement in the U.S. population.

Significance to Biomedical Research and Program of the Institute: The study is relevant to the program of research in the behavioral sciences because it deals with one extreme of the fertility distribution curve, and can shed light on factors affecting motivation for fertility limitation. It is particularly timely in view of current social emphases upon fertility limitation and advocacy by some groups of childlessness and one-child families. The study of factors affecting such behavior provide complementary data to the behavioral science program to study consequences of family size.

Proposed Course: This contract is expected to be completed during FY 79.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : How Mother's Age and Circumstances Affect Children
Contract Number : NO1-HD-82806
Contractor : University of Southern California
Money Allocated : $95,058 (1978)

Objectives: This is a two-year study which makes use of sample data that were collected in 1963-65 in the Health Examination Survey conducted by NCHS in cooperation with the Bureau of the Census. The sample included children 6 to 11 years of age in a representative nationwide survey who were administered physical and psychological tests, whose parents and teachers provided background information and evaluations, whose records in the offices of their physicians, dentists, and hospitals were consulted, and whose birth certificates are to be obtained. This information is available for about 7000 children, 300 of whom can be identified as illegitimate. The purpose of the study is to determine whether youthful age of mother and growing up in a disrupted family adversely influences the development of the child, controlling for social class and birth order.

Major Findings: It has been found that there is an inverted U-shaped relationship between mother's age and the child's (10 and 11 year olds) intellectual and educational development. It may be inferred from the findings that the optimum age for a mother to have a child is between 23 and 29. Children's intellectual performance is lower if the mother is a teenager. Children of teenage mothers do significantly more poorly in school if they are also financially disadvantaged. Mothers working full time, more educated mothers, and mothers who breastfeed have children who are less likely to fail in school.

Significance to Biomedical Research and Program of the Institute: This research is relevant to the Institute's interests in the correlates and consequences of fertility.

Proposed Course: It was expected that this contract would be completed in September, 1979, but a nine month extension has been requested.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : The Consequences of Family Structure and Maternal State for Child and Mother's Development
Contract Number : NO1-HD-82807
Contractor : University of Southern California
Money Allocated : $174,357 (1978); $98,849 (1979)

Objectives: This is a study of the effect of family size and childspacing upon the health and social development of children, using a Danish population of 9000 children born in 1959-61 in Copenhagen. Data will be analyzed to determine effects of order of birth and sex, age of mother, wantedness, spacing, perinatal factors, parental health and social and family structure variables. Additional data will be collected on development of children, school performance, delinquency, and psychological and social functioning.

Significance to Biomedical Research and Program of the Institute: This study will exploit an excellent data file to explore the consequences of family size, birth order, and childspacing for a variety of health, psychological, and social outcomes. It promises to contribute considerably to knowledge concerning the effect of both family size and birth spacing on the health and social development of children.

Major Findings: In this study, a linear relationship was found between mother's age and pre-, peri-, and neo-natal problems, with teenage mothers experiencing the fewest complications. Similarly, no relationship was found between negative neonatal outcomes and social risk factors, such as low SES, illegitimacy, and unwantedness of pregnancy. These findings corroborate results of the American Collaborative Perinatal Study where University hospitals, which provided high quality health care, were also used. They contradict, however, the findings of most population studies involving more representative populations in the U.S., Britian and Denmark. The results were interpreted as an indication of the importance of adequate medical and health care, particularly for young and low SES mothers. Pregnancy complications, delivery complications, and birthweight were found to be the most significant predictors of infant survival and neonatal health. While low birthweight is the most important predictor of infant survival and health, none of the medical and social variables included in the study predicted birthweight.

Neurological functioning and motor development at one year of age appear to be associated with medical and biological factors. However, physical health at one year is related to social factors. Children of low SES and children of unmarried mothers who live alone with their children generally show poorer physical health. The study also finds that children of older mothers, 25 years and up, do better than children of younger mothers, except in cases where teenage mothers rely upon older women (i.e., grandmothers) for child care.

Proposed Course: This study is a continuation of a project initiated in FY 76 at Columbia University and is expected to be completed in two years.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Psychological Factors and Fertility Regulation in Women: Data Analysis
Contract Number: NO1-HD-8-2831
Contractor : American Institutes for Research, Palo Alto, California
Money Allocated: $110,569 (1978)

Objectives: This study will analyze extant data collected by the Principal Investigator in a study of psychological and behavioral factors affecting fertility regulation in women. Data were gathered annually over a four year period for three main subject groups: 325 never-married women, 322 just-married women, and 320 just mothered women (having their first child). The study sample was of restricted age and marital status, all fecund, white, and English-speaking women, selected randomly from a specified urban/suburban area around Stanford, California. Semi-structured interviews and self-report questionaires focused on psychological and behavioral aspects of (a) cohabitation, marriage, sexuality, contraception, gender role activities, occupational activities, and divorce as each of these domains affected fertility, and on (b) proception, childbearing, and childrearing. The present study will analyze the data to: (1) test the adequacy of a general causal model of individual fertility regulation behavior; (2) examine in detail the relationships between categories of behavior that may affect fertility regulation; (3) test hypotheses about the effect of specific psychological traits and behaviors on fertility regulation; and (4) develop descriptive information about various aspects of the fertility regulation sequence.

Major Findings: Preliminary findings show that in intact marriages, proception (behavior intended to achieve conception) is by far the most important determinant of the occurrence of conception, with the woman's fecundity, the type of contraceptive method she uses, and the frequency or adequacy of contraceptive use being equally but far less important, and with frequency of sexual intercourse not being important at all apart from the very few women who abstain. The most important determinants of proception are the length of time since the last child was born and how soon the next child is expected. The most important determinants of how soon the next child is expected are how many (additional) children are expected and the length of time since the last child. A woman's working affects childbearing primarily by delaying or preventing proception and by increasing the length of time before she expects to have her next child. Marital satisfaction and cohesion affect childbearing primarily by promoting proception, by decreasing the length of time before she expects her next child, by increasing the number of (additional) children she expects to have, and by decreasing the woman's election of an induced abortion when she experiences an unplanned pregnancy.

Significance to Biomedical Research and Program of the Institute: This research is relevant to the Institute's interests in the correlates and determinants of fertility regulation behavior.

Proposed Course: This contract is expected to expire December 31, 1979.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : American Attitudes Toward Abortion
Contract Number : NO1-HD-8-2832
Contractor : University of California at Los Angeles
Money Allocated : $92,668 (1978)

Objectives: Building on the Principal Investigator's research on abortion attitudes over the past 12 years, this study analyzes existing data and generates new data on abortion attitudes over a three-year period as expressed in U.S. national samples interviewed by the Gallup Poll. The emphasis is on demographic and social-psychological correlates of attitudes toward various aspects of the abortion issue. The two phases are interrelated. The first emphasizes descriptive, socio-demographic and locational aspects of abortion attitudes, including changes over time. The second major component studies sociological and personality predictors of abortion attitudes. The project utilizes Gallup survey data going back to 1962, new data to be collected during the course of the contract, data from the National Opinion Research Center (NORC) surveys, and information from the 1965 and 1970 National Fertility Surveys (NFS). Disaggregation of abortion attitudes as the dependent variable--timing of abortion, belief about when life or personhood begins, government aid for abortion, etc.--appears to be increasingly significant as people become more aware of the complexity of the abortion problem.

Major Findings: An analysis of previous survey questions on the conditions under which people regard induced abortion as justified (mother's health, birth deformity, economic stress, and elective abortion) reveals that the most important distinction is between whether people disapprove abortion for all reasons or approve for all reasons and not whether they express approval or disapproval according to graded justifications. The investigator is also finding what appears to be the development of more clear-cut ideological differences among people belonging to the various Protestant denominations. In some cases, these differences appear to be even greater than the Catholic- non-Catholic differences in views toward induced abortion.

Significance to Biomedical Research and Program of the Institute: This research, requested under RFP 78-6 relating to "the regulation of fertility," is central to the Program of the Center and is highly relevant to an understanding of the determinants of choice among various methods of fertility control.

Proposed Course: Additional surveys will be conducted over the next two years and the project is scheduled to be completed in FY 81.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Psychological Factors Associated with Fertility Regulation: A Comparison of Pregnant and Non-Pregnant Adolescents
Contract Number : NO1-HD-82833
Contractor : The Philadelphia Health Management Corp., Philadelphia, Pa.
Money Allocated : $85,585 (1978)

Objectives: The primary purpose of this study is to examine the psychological/thinking processes operating when a female adolescent does or does not become pregnant. A secondary purpose is to conduct an exploratory study of the knowledge and attitudes toward contraception and attitudes toward teenage fatherhood of male adolescents. The design of the project is based on the belief that while studies of structural and demographic variables are valuable for their identification of high-risk groups, they do not provide understanding of the thinking processes at work. Without such an understanding, it is believed that intervention programs cannot be developed which specifically meet the needs of these adolescents.

The subjects for this study will consist of female and male black urban adolescents of lower SES. Three age matched samples will be included in the study. The first will consist of 90 adolescents entering their third trimester of pregnancy who have volunteered for one of two alternative programs run by the Philadelphia School District: these adolescents will be interviewed once, as they enter the program. The second sample will be a comparison group of 180 nonpregnant adolescents, matched (on a 2 to 1 basis) with the first on age, residential location (as an indicator of SES) and race. These adolescents will be interviewed twice; at the initial interview they will not be pregnant and they will be interviewed one year later, when it is expected that approximately 25% will have become pregnant during the past year. The third sample will consist of 90 male black adolescents who have volunteered for a school district group discussion series on sexuality.

The interview with the adolescents will consist of a fact sheet, three standardized instruments assessing elements hypothesized to be essential to effective contraceptive use: a sense of control over one's own life, the ability to anticipate the steps for reaching a goal, and the ability to anticipate consequences. At the follow-up interview with the comparison group, the interview will consist of: the fact sheet; the instrument measuring sense of control; and questions about knowledge, attitudes, and information sources about contraception, and questions about expectations for work, family and relationships.

Major Findings: There are no findings to report at this time.

Significance to Biomedical Research and Program of the Institute: This research is relevant to the Institute's interests in the correlates of adolescent fertility regulation behavior.

Proposed Course: This contract is expected to be completed in March 1980.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : A Multifaceted Analysis of Abortion as a Social Issue in the United States, 1965-1978
Contract Number : NO1-HD-82834
Contractor : University of Missouri
Money Allocated : $45,714 (1978)

Objectives: The purpose is to analyze abortion attitudes in the U.S. between 1965 and 1978. Available national survey data obtained from the National Opinion Research Center (1965, 1972-1977) and the Survey Research Center (1972 and 1976) are being used to describe the climate of opinion and changes over time; to test 37 hypotheses regarding attitudes to abortion; and to integrate the findings so as to enhance the understanding of psycho-social issues underlying abortion attitudes. Among the variables being considered in relation to abortion attitudes are socio-economic indicators including education, political ideology, religion and views on women's liberation and human rights.

Major Findings: Attitudes toward making legal abortion available became much more favorable between 1965 and 1973 and did not change until 1978, when there was a small but significant decrease in approval of abortion for social-psychological reasons. Education was the socio-economic status indicator most strongly correlated to abortion attitudes, with approval of abortion increasing with the amount of formal education. A slight tendency was found for political conservatism to be correlated with disapproval of legal abortion, especially among people with some college education. Abortion attitudes were consistently and strongly linked to conservatism regarding matters of personal morality. People disapproving of abortion being legally available were also likely to disapprove of premarital and extramarital sex, homosexual relations, sex education, birth control information being available, marijuana, lenient divorce laws, and permissiveness in regard to pornography. Jews and those without religious affliliation were the groups most approving of legal abortion. Among Protestants, Episcopalians were the most approving, and Baptists the least approving. Catholics were consistently more opposed than Protestants. Protestants, and to a slightly lesser degree Catholics, who favor gender equality are more likely to approve legal abortion.

Significance to Biomedical Research and Program of the Institute: Each year over one million women terminate pregnancies by means of a legal abortion. This research addresses factors relating to this major health and fertility issue.

Proposed Course: This project began in FY 78.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : A Proposal to Investigate the Determinants of Variations in U.S. Fertility Trends 1940-1975
Contract Number: NO1-HD-8-2835
Contractor : Mathematica Policy Research, Inc.
Money Allocated: $102,443 (1978); $42,356 (1979)

Objectives: This project seeks to determine how economic trends during the period 1940-1975 have affected the trend in fertility. Changes in both timing of births and total family size will be related to cyclical variations in female labor force participation and government income transfer payments, as these affect and are affected by changes in relative income.

Major Findings: A preliminary model of the relative importance of changes in the timing of fertility and changes in desired completed fertility has been developed. Preliminary results from cohort fertility regressions suggest that the effect of relative income on cohort fertility is positive and significant. Relative income is defined in the Easterlin context as the ratio of a cohort's expected lifetime income to the expected income of its parental generation. When this ratio is separated into two variables, the results indicate that increases in a cohort's expected lifetime income induces earlier childbearing, but that increases in the parental generation's lifetime earnings has no significant effect on the timing of its offspring's fertility.

Significance to Biomedical Research and Program of the Institute: This project is relevant to the program of the Center dealing with social and economic factors which have influenced wide fluctuations in the birth rate since the early 1940's, and will contribute insights into the role of government policies in affecting the level of fertility.

Proposed Course: This project was initiated in FY 78 and is expected to be completed in FY 80.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Adolescents' Use of Information about Conception and Contraception
Contract Number : NO1-HD-82836
Contractor : Planned Parenthood Association/Chicago
Money Allocated : $91,224 (1978); $61,419 (1979)

Objectives: Fifteen adolescent couples from each of five Chicago Area Teen Scene Clinics will be chosen from different ethnicities and by quotas within each clinic to obtain five seeking contraception, five who are pregnant, and five with a negative pregnancy test or "pregnancy scare." In addition, at the end of one year, 10 female adolescents from each of the five clinics will be interviewed as controls. Data will be gathered by means of clinical interviews and from journals kept by the young people. Topics include the adolescents' information about contraception, conception and sexuality, and their use of that information in the context of their sexual, contraceptive, and pregnancy behaviors. Based on Piaget's theoretical work, the investigator argues that mere assessment of knowledge levels among adolescents concerning contraception and other sexual matters is insufficient to understand the role of that knowledge in behavior--we should get behind correlations into the cognitive processes. Adolescents assimilate knowledge selectively and then use if differently depending on their own contexts.

Major Findings: Early interviews show, especially among younger adolescents (ages 13-15), that their reproductive anatomy and physiology, their thinking and experience with conception, contraception, and heterosexuality are experienced as pieces of a complex puzzle that do not fit together and are only dimly perceived. Females are far more concerned and knowledgeable about conception and contraception than are their male partners, yet their effective use of contraceptive methods is compromised by incomplete understanding of how they work and by notions that certain methods will not work for them. The difficulty which some experience with defining the nature and beginning of their menstrual periods affects their use of oral contraceptives and the early determination of pregnancy. This confusion probably associated with irregular menstruation in early adolescence, becomes resolved with the experience of oral contraceptive use or of pregnancy.

Significance to Biomedical Research and Program of the Institute: This research, requested under RFP 78-8 relating to "the determinants of adolescent pregnancy and childbearing," is central to the program of the Center and is highly relevant to departmental initiatives and to Congressional interest, as there is considerable question about the effectiveness of present modes of presenting such information to adolescents.

Proposed Course: This project, initiated in FY 78, is scheduled to be completed in FY 80.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Adolescents' Perspectives on the Health Care Systems: A Determinant of Fertility
Contract Number : NO1-HD-82837
Contractor : University of North Carolina
Money allocated : $105,786 (1978); $108,120 (1979)

Objectives: The purpose is to identify the nature and source of adolescent women's perspectives on the established medical care system, and the impact of these perspectives upon their use of the system for fertility-related services. The objectives of the study are: (1) to identify the specific reasons that may make adolescent women reluctant to use the established medical care system for fertility-related services, and (2) to assess the usefulness of a variety of data collection techniques for use with adolescents.

A variety of methods will be used to obtain a comprehensive list of adolescents' concerns regarding medical care. A typology will be developed along with a conceptualization that is in empirically verifiable terms. The research will involve unstructured group and individual interviews, in formats determined by a Research Advisory Panel (RAP) of adolescents, to obtain information from 200-300 adolescent women. A variety of techniques will be used to draw the adolescents' attention to the topics under consideration. At the end of the project the total data file will be analyzed and inductive techniques utilized to develop conclusions, recommendations and guidance for further study. Findings will provide (1) bases for further research and (2) guidelines for improving medical services for adolescent women.

Significance to Biomedical Research and Program of the Institute: Other studies have pointed to the high proportion of adolescent contraceptors who use clinic services, but little research has addressed the teenagers' failure to use the established medical care system, i.e., permanent broad spectrum institutions. This wide-ranging medical system may be the only one large enough to meet the demands of adolescents for family planning and related services. This research fills a gap in the research program on adolescent fertility behavior.

Proposed Course: This project was begun in FY 78 and is scheduled for completion in March, 1981.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Research on the Determinants of Repeated Adolescent Pregnancy and Childbearing
Contract Number : NO1-HD-8-2838
Contractor : Johns Hopkins University
Money Allocated : $52,752 (1978)

Objectives: This project will use the extant data base of a Johns Hopkins longitudinal research and service program established in 1973 for adolescent mothers to identify those young women who had a subsequent (unwanted) pregnancy and compare them with matched controls who did not experience a subsequent pregnancy. This study will include about 90 cases and an equivalent number of controls matched by race and age, and will consist primarily of data collected during the first pregnancy and at the four-week postpartum visit. The data available about mother, father and pregnancy should include for each pregnancy demographic, educational and family background variables, contraceptive usage, amount of prenatal education, contraceptive prescribed postpartum, etc. They should provide: (1) a broad and complete picture of the problem among these urban adolescents; (2) gross indicators of risk of repeated pregnancy; and (3) outcome of subsequent pregnancy.

Major Findings: Since the research has been under way only a few months, not manyresults are available. Among 225 girls followed 24 months, 6% had a repeated pregnancy in 12 months and 13% within 24 months of the index delivery. This is a low percentage within one year as compared with approximately 25% on a national level and 47% in this same population at an earlier time.

Significance to Biomedical Research and Program of the Institute: This research, requested under RFP 78-8 relating to "the determinants of adolescent pregnancy and childbearing", is central to the program of the Center and is highly relevant to departmental initiatives and to Congressional interest in this area. The project's considerable value lies in the study of a segment of the population that creates hardships for themselves and society--adolescents with repeat pregnancies. It could lead to predictive data about individual characteristics as well as the identification of structural sources of repeat pregnancy for which immediate solutions can be devised.

Proposed Course: This project is scheduled to be completed in September 1979.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Determinants of Adolescent Sexuality, Contraceptive Use, and Pregnancy Resolution
Contract Number : NO1-HD-8-2839
Contractor : Transnational Family Research Institute
Money Allocated : $77,561 (1978)

Objectives: The purpose is to explore factors influencing sexual behavior, contraceptive usage and pregnancy resolution among sexually active black urgan adolescent men and women under age 18. Experienced peer counselors in six high schools in Washington, D.C. will be queried about their observations with sexually active students, particularly males. Topics to be explored include: (1) sex education; (2) parental influence and home environment; (3) peer pressure; (4) media influence; (5) characteristics of the couple relationship; and (6) academic achievement, career aspirations and perceived job opportunities. Also to be explored are reasons for using available family planning services, carrying an unintended pregnancy to term or seeking an abortion, marriage, and keeping an out-of-wedlock child or placing it for adoption. The main criterion in selecting topics will be to include only those to which peer counselors can respond on the basis of their observations of peers.

Major Findings: Preliminary tentative findings suggest the following classification of determinants of teenage pregnancy:

A. Lack of parental guidance and supervision,
B. Peer influence,
C. The taboo status of interpersonal communication concerning sex and contraception,
D. The macho sterotype among black adolescent males,
E. The unacceptability of available contraceptive methods and inadequate means of distribution, and
F. Ignorance about the risks of pregnancy.

Significance to Biomedical Research and Program of the Institute: This research will contribute to our understanding of contraceptive practice among adolescents. The increasing sexual activity among adolescents and the frequently poor use of contraceptives make this a topic of considerable significance.

Proposed Course: This one-year project will end in September 1979.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Sociosexual Development in Black Adolescents within the Family Context: A Longitudinal Study
Contract Number: NO1-HD-8-2840
Contractor : Howard University Hospital
Money Allocated: $99,499 (1978); $107,253 (1979)

Objectives: The purpose is to explore the effects of psychological adjustment, sexual knowledge and attitudes, family interaction, moral and ethnical reasoning, socio-economic status and environmental impact on sexual behavior. The project involves a five-year longitudinal study of 160 black early adolescents and their parents. Periodic medical examinations, unstructured interviews using a variety of stimulus materials, and videotaping of family interaction will be used. Participants will be a volunteer non-responsive sample of healthy 10-year-olds from low and middle income families, evenly split male and female. Test materials (pictures, incomplete stories and simulated taped conversations) will be developed. Some family interaction observations will be made.

Significance to Biomedical Research and Program of the Institute: This study addresses the social and physiological changes around adolescence relating to a sexual development. Concern about adolescent sexual and reproductive behavior is shared by biomedical and social scientists. This proposal addresses a critical junctive of the two fields on an issue of considerable importance.

Proposed Course: This project was begun in FY 78 and will terminate in FY 81.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : An Analysis of the Determinants of Adolescent Pregnancy and Childbearing
Contract Number: NO1-HD-8-2841
Contractor : Mathematica Policy Research, Inc.
Money Allocated: $83,695 (1978); $49,120 (1979)

Objectives: The purpose is to develop and estimate a model of adolescent fertility which incorporates the interdependencies among teenage sexual experiences, contraceptive use, pregnancy and childbearing. The analysis proposed will include two parts: (1) an analysis of the social, economic and biological determinants of the decision to engage in sexual activity, use of contraceptives, probability of pregnancy and the outcome of pregnancy; and (2) an increment-decrement life table analysis based on the empirical results from part 1. The data to be used for both parts 1 and 2 come from the 1971 Kantner and Zelnik study of teenage sexuality and fertility.

Major Findings: A preliminary model has been developed of the relationship between four components of adolescent sexual behavior and a set of independent variables measuring the socio-economic background characteristics of adolescent women. Using data from a 1971 national probability sample of U.S. females aged 15-19, the four main dependent variables are sexual activity, contraceptive use, pregnancy, and pregnancy outcome. The results to date are for the sexual activity decision and suggest that family structure variables are significant predictors of the probability of intercourse. Teenage women who grow up in a household with both a male and female parent are less likely to be sexually experienced than adolescents in a single-parent household, after controlling for age, religion, and various other socio-economic characteristics. Educational expectations have a significant negative effect on the probability of intercourse, as do father's education and the age of the household head. An interesting result concerns the labor supply variables. Teenage women who work and go to school are less likely to be sexually experienced, but among the subsample of sexually active adolescents, work and school appears to be associated with a younger age at first intercourse.

Significance to Biomedical Research and Program of the Institute: Adolescent pregnancy and childbearing is a topic of concern for biomedical researchers and social scientists since the effects of early reproduction are felt in both areas. This project will address the role of biological and social factors relating to key reproductive events.

Proposed Course: This two-year project was begun in FY 78 and will terminate in FY 80.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Contraceptive Use-Effectiveness: The Fit Between User and Method Characteristics
Contract Number : NO1-HD-82842
Contractor : Claremont Graduate School
Money Allocated : $49,492 (1978)

Objectives: An intensive archival study of current and past contraceptive clinic records will investigate the use-effectiveness of various contraceptive methods for specific subgroups of the teenage clinic population on the relationship between method characteristics and user characteristics in determining success or failure in contraception. The data set covers 800 teenage contraceptors. The teenage clinic participants were given extensive personality tests, KAP questionnaires and interviews at clinic intake and were followed for three years. One-seventh became pregnant, and others dropped out, changed method, or had various contraceptive use-effectiveness outcomes. Use effectiveness outcome variables other than unwanted pregnancy will be related to earlier personality, knowledge and attitude measures to determine the degree to which prior knowledge, attitudes, and personal characteristics influence later use-effectiveness of contraception. Theoretical relationships to particular methods have already been established.

Major Findings: Preliminary, tentative findings show that as far as contraception related side effects are concerned, few differences were found between patients based on age, ethnic background, marital status, weekly household income, or highest grade completed. However, teenagers who experienced more side effects were significantly less modern, more feminine, and lower in heterosexual initiative and in avoiding suffering. Interviewers perceived them as having a more negative self-concept and more feelings of inadequacy; they were rated negatively on most of the personal characteristics.

Significance to Biomedical Research and Program of the Institute: This research, requested under RFP 78-8 relating to "the determinants of adolescent pregnancy and childbearing", is central to the program of the Center and is highly relevant to departmental initiatives and to Congressional interest in this area. It has direct and immediate applications at the service delivery level and should give us a valuable start (but not parameters) in an area on which we know very little.

Proposed Course: This project is scheduled to be completed in December 1979.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Policy Determinants of Teenage Childbearing
Contract Number : NO1-HD-82843
Contractor : The Urban Institute
Money Allocated : $64,253 (1978) $30,657 (1979)

Objectives: The purpose is to assess the relationship of various state and federal programs to teenage childbearing. The programs presumed to have a direct effect are family planning services, abortion availability, age of consent laws concerning contraception and abortion, Medicaid coverage of abortion, welfare benefit levels and acceptance rates, coverage of unborn children and unemployed fathers under welfare programs, and benefit levels in food stamp and Medicaid programs. Indirect policy influences include local unemployment rates, female wages, and educational opportunities. State information on these programs and policies will be added to the 1976 public use tape of the Survey of Income and Education (SIE). This survey provides data on fertility, demographic, and family characteristics of an estimated 30,000 females aged 14 to 19. Two sets of analyses are proposed: (1) characteristics of individual females, plus state-level policy and control variables, will be regressed on whether a female aged 14 to 19 has a child under one year of age; and (2) state-level policy variables plus state-level control variables will be regressed in teenage fertility rates in each state.

Significance to Biomedical Research and Program of the Institute: This research addresses a topic of considerable importance and interest in both biomedical and social science areas--adolescent reproduction. Specifically, the influence of health policies (medicaid coverage, abortion availability, etc.) will be assessed.

Proposed Course: This 18 month project was begun in Fiscal Year 1978.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Exploration of the Effects of Organized Family Planning Programs in the United States on Adolescent Fertility
Contract Number: NO1-HD-8-2844
Contractor : The Alan Guttmacher Institute
Money Allocated: $74,546 (1978); $56,601 (1979)

Objectives: Using extant data ftom a variety of sources, this study will assess the impact of family planning clinic programs in the United States on adolescent (15-19 years of age) fertility of both black and white women. A variety of measures of social, demographic and family planning service characteristics will be compiled for Statistical Analysis Units (counties or clusters of counties) and then subjected to multivariate techniques of analysis to answer two principal questions: (a) what is the association between the level of teenage fertility and the availablility and teenage utilization of family planning clinic services, and (b) what is the association between changes in the availability and teenage utilization of family planning clinic services and changes in the level of teenage fertility in the period 1970-76? The multivariate analysis will seek to control a wide range of other determinatnts of the level of teenage fertility and of the impact of abortion and private physician services as alternative family planning elements. Data sources include: (1) county-level data from special tabulations of the 1970 Census to provide 1970 fertility rates for women 15-19 by race as well as family planning program data for 1969 and socioeconomic and demographic control variables for 1970; and (2) newly available data for 1975 and 1976. This study is largely an extension of an earlier project (Cutright and Jaffe) sponsored by the Alan Guttmacher Institute and supported by the Center for Population Research, NICHD, under Contract NO1-HD-32722. Results were published in Impact of Family Planning Programs on Fertility: The U.S. Experience (Praeger, 1977, 150p.).

Significance to Biomedical Research and Program of the Institute: This research requested under RFP 78-8 relating to "the determinants of adolescent pregnancy and childbearing," is central to the program of the Center. While the study is essentially an evaluation of family planning clinic services to teenagers, and thus highly focused on one determinant of adolescent fertility, it is of importance for the policymaker and program expert to answer the general question of whether differential availablility of and enrollment in organized family planning program offering highly effective medical methods of contraception to teenage women had a measurable impact on their fertility. The study design should provide indirect evidence of the impact of family planning clinic services by presenting areal or ecological relationships between the variables.

Proposed Course: This project, underway in FY 78, is scheduled to be completed in FY 80.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Psychosocial Determinants of Adolescent Contraceptive Use
Contract Number : NO1-HD-82847
Contractor : The George Washington University
Money Allocated : $31,218 (1978)

Objectives: This research is an attempt to identify psychosocial and other determinants of adolescent sexual activity, contraceptive use and pregnancy. The psychosocial factors include a number of sex- and contraceptive-related attitudes, sex and contraceptive knowledge, social experience, personality traits, social and environmental pressures, future aspirations and prior history of sexual experience. An American Public Health Association (APHA) survey of 835 16-18 year old males and females, containing detailed psychosocial information, will be used. Also available for analysis is 30-month longitudinal information on a sub-sample of about 200 of the original respondents. The longitudinal sample can be used to trace causal relationships between psychosocial factors and fertilityrelated behavior. Since males as well as females are included, the role of psychosocial factors on parenthood can be compared. Analysis will be undertaken through three inter-related phases focusing on the psychosocial determinants of: (1) sexual behavior, (2) contraceptive behavior, and (3) pregnancy. Demographic and social class data will be examined for their moderating effects on these major relationships.

Major Findings: Sexual Behavior: Various liberal attitudes about sex (i.e., approval of intercourse) are the most powerful differentiators of virgins from nonvirgins. Other statistically significant findings include nonvirgins being more self-confident, perceiving a greater availability of contraception and need for its use, and being more traditional in viewing male-female roles. Virgins perceive more negative features to contraception and are more embarrassed in discussing it. Contraceptive Behavior: Effective contraceptive users perceive contraception as available, and less effective users perceive more negative features, are embarrassed by contraception, and are more willing to risk conception. These relationships are stronger as static rather than over-time covariations, and causal direction is not clear.

Significance to Biomedical Research and Program of the Institute: This research, requested under RFP 78-8 relating to "the determinants of adolescent pregnancy and childbearing", is central to the Center program. It attempts to give a good, first look at little known factors, e.g., whether a person feels that lack of access to contraceptive services is a problem. Two outputs--ability to assess causal relationships in a longitudinal framework and parallel analysis for male and female teenagers--make this project original and potentially very useful for public policy. The analyses will be examined for findings relevant to the formulation of policy and intervention strategies.

Proposed Course: This project is scheduled to be completed in September 1979.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Determinants of Fertility Behavior Among U.S. Females Aged 15-19, 1971 and 1976
Contract Number : NO1-HD-8-2848
Contractor : The Johns Hopkins University
Money Allocated : $109,576 (1978); $116,951 (1979)

Objectives: The purpose is to analyze the relationship of various facets of reproductive behaivor to several clusters of background and contextual variables which are believed to have a determining influence. These variables include: family background, relative influence of parents and peers, religion, residence, and residency history, future orientations relevant to marriage, childbearing, education and career, perception of normative situations regarding illegitimacy and abortion, selected situational variables such as employment, social life and dating behavior.

Data to be used for this analysis come from two national surveys conducted five years apart in 1971 and 1976. These surveys, funded through a grant from NICHD, include information on sexual activity, use of contraception, pregnancy and the resolution of pregnancy. Each of these aspects of behavior which can be treated as a dependent variable will be dealt with in finer detail in the analysis. The analysis will take the form of two-way cross tabulations controlling for race and Multivariate Analysis.

Major Findings: Although the 1971 and 1976 studies show that the use of effective methods of contraception has increased among unmarried teenage girls, there are still many who do not use contraception. The major reason given for not using contraception is the belief that pregnancy could not occur--for a variety of reason, including having intercourse at the time of the month when it was thought that pregnancy could not occur, being too young to become pregnant, and having intercourse too infrequently to become pregnant. Another important reason for not using contraception was that the girl had not expected to have intercourse. The study also reveals that 1 in 5 girls have had intercourse by age 16 and two-thirds by age 18. One in 10 become pregnant before age 17, and one-quarter before age 19. Of those pregnancies, 80% are premarital. Among those teenage girls who are sexually active before marriage, one-quarter become pregnant before reaching age 17, and over one-third before reaching age 19.

Significance to Biomedical Research and Program of the Institute: Adolescent reproduction is a topic of concern to a broad spectrum of scientists as well as the public and policymakers. This study is based on the best sources of data for the U.S. as a whole, on this topic. This analysis assesses important aspects of sexual and contraceptive behavior.

Proposed Course: This 2-year project will be completed in FY 80.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Social and Cognitive Development of Only and First Born Children
Contract Number : NO1-HD-8-2849
Contractor : Educational Testing Service, Princeton, New Jersey
Money Allocated : $92,576 (1978); $95,844 (1979)

Objectives: Using existing data from a longitudinal study of children, their mothers and mother-child interaction at 3, 12, 24, and 36 months of age for the child, longitudinal and cross-sectional analyses will contrast behaviors and cognitive levels of only and first borns at four ages; will measure the impact of acquiring a sibling at two ages (before or after 24 months of age); and will contrast planned and unplanned children on the measures taken. In addition, the decision to have a second child will be examined from the perspective of the mother's locus of control, femininity, concept of ideal family size, and quality of interaction with the first child.

Major Findings: Preliminary findings from the data at each age level reveal that only children whose parents are planning to have more children perform consistently higher on tests of cognitive functioning than onlies whose parents are not planning to have additional children. Maternal stimulation and interaction are currently being explored in an attempt to determine the role of these factors in the differential performance of children who will remain onlies and children who will most likely not remain onlies.

In the case of children who acquired a sibling during the course of original data collection, the data reveal that the age at which first-born children acquire a sibling is related to their cognitive performance. The experience of the birth of a sibling before age 2 years is related to decreases on the Bayley MDI as compared to performance before this event. However, the MDI scores of children acquiring a sibling after age 2 and before age 3 remain stable.

Significance to Biomedical Research and Program of the Institute: This research is relevant to the Institute's interests in the correlates and consequences of fertility.

Proposed Course: This contract will take two years to complete, and will continue into FY 80.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : The Consequences of Remaining Childless or Having Only One Child: An Analysis of Selected Outcomes for Husbands & Wives
Contract Number : NO1-HD-82850
Contractor : The Urban Institute
Money Allocated : $88,267 (1978) $89,107 (1979)

Objectives: The purpose of this study is to analyze the consequences of family size, specifically zero and one-child families in relation to families with more children. The issues to be addressed are: (1) economic well-being (income, ratio of income and needs, assets, expenditures, debts); (2) time availability (hours spent in the labor force, housework, childcare); (3) schooling, training and experience (labor force experience acquired, education and training completed); (4) geographic mobility (moved within or across boundaries of various types: counties, states, regions); (5) marital stability; and (6) health (limitations on work or activities in general). Besides the particular number of children, a focus of this research will be timing of births within the marital cycle. Since it is expected that the consequences of different family sizes for couples and never-married individuals will depend on their stage in the marital cycle (and their age), the outcomes of interest will be examined among couples and individuals who are comparable in terms of length of time since first marriage. Finally, the impact of adding a child to the family will be examined among couples at different stages in the marital cycle. The analysis will be based on the Michigan Panel Study of Income Dynamics, PSID. The PSID followed heads of families, wives and their families over nine years, 1968 to 1976 inclusive and contains a wide array of information.

Significance to Biomedical Research and Program of the Institute: As increasing numbers of couples are electing to have none or one child, the consequences of such behavior are of growing interest and concern. This study will assess both the social and health consequences of very low fertility.

Proposed Course: This two year project was begun in Fiscal Year 1978.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Studies on the One-Child Family
Contract Number : NO1-HD-82851
Contractor : University of California, Berkley
Money Allocated : $91,452 (1978), $69,592 (1979)

Objectives: The purpose of this project is to compare only children and children with siblings on a variety of physical and behavioral measures. Data consists of records of children enrolled in the Kaiser Health Plan collected at the time of the mother's pregnancy, and when the child was 5, 10, and 15 to 17 years of age. The records included information of the mother's reaction to the pregnancy, child's physical growth, cognitive abilities, behavioral characteristics, and prospective data on "intendedness" of future children, and contraceptive use. Consequences of being an only child (compared to being oldest and youngest in two and three child families) shall be analyzed and the determinants of the one-child family shall also be described. Racial differences shall also be assessed to the extent feasible.

Major Findings: Preliminary analyses have found no substantial difference between only children and the oldest of two or three children. There is, however, one substantial difference in the family of the only child: the mother is more likely to work full time. This preliminary finding must be regarded with caution, though, as the comparison mothers have a five-year-old and one or two younger children at home.

A very preliminary conclusion is that several of the special characteristics often ascribed to single children by and large disappear if confounding variables are controlled. This supports Falbo's work that exposes the untenability of the folklore on the only child.

Significance to Biomedical Research and Program of the Institute: This study is relevant to the Institute's interests in the determinants and consequences of fertility.

Proposed Course: This study is expected to continue into FY 80.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : The One-Parent/One Child Family: Sociological and Psychological Consequences
Contract No. : NO-HD-8-2852
Contractor : American Institutes for Research in the Behavioral Sciences
Money Allocated: $47,235 (1978) $71,496 (1979)

Objectives: The aim of this study is to make an exploratory examination of the structure, functioning, and interpersonal dynamics of a family form that includes a mother and child in a two-person family and compares this family form with three other family forms: the two parent/one child family; the one-parent/two child family and the one-parent/three child family. The families in the study shall be divided among those with children from age six or under to those with children up to 18 years old. Clinical, exploratory interviews will be conducted with 100 women and with their children (in the adolescent subsample). The focus of the study will be on the mother in terms of her work, leisure, economic well-being, educational pursuits, and perceptions of parental role, and on the child in terms of household responsibilities, maturity, life-satisfaction, use of time, future goals, and psychological well-being.

Significance to Biomedical Research and Program of the Institute: This research is relevant to the Institute's interests in the correlates and consequences of fertility.

Proposed Course: This research will take 18 months to complete and will continue into FY 80.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title	:	The Personal Meaning of Voluntary and Involuntary Childlessness
Contract Number	:	NO-HD-82853
Contractor	:	American Institutes for Research in the Behavioral Sciences
Money Allocated	:	$47,981 (1978)

Objectives: This study examines the antecedents to and personal meanings of childlessness within a marriage. In-depth psychiatric interviews will be conducted with individuals from 60 intact marriages. In one-half of the marriages the age of the wife shall be 35-40; in the other, the age shall be 50-55. Within each age group, one-half of the couples shall be childless by choice, and one-half childless as a consequence of an incapacity to conceive or bear children. A composite picture of the main pathways through which married individuals and couples arrive at a childfree state shall be drawn from analysis of in-depth interviews, and a picture presented of the personal meanings of this state near the end of the reproductive period and during the post-reproductive period shall be drawn. In addition, a descriptive account of the life course of childfree individuals and couples shall be developed. Hypotheses for future research shall be generated after analysis of the findings of this study.

Significance to Biomedical Research and Program of the Institute: This study is relevant to the Institute's interests in the antecedents, determinants, and consequences of fertility.

Proposed Course: This study is expected to continue into FY 80.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : The Consequences of Being an Only Child: An Analysis of Project TALENT Data
Contract Number : NO1-HD-82854
Contractor : American Institutes for Research in the Behavioral Sciences
Money Allocated : $85,669 (1978)

Objectives: Using existing data from Project TALENT, (a longitudinal study of high school students in 1960 with subsequent data collection 11 years later) the researchers are investigating the consequences of being an only child. Comparisons will be made between only children and their cohorts. The investigators, using a model developed from previous research findings, predict that birth status relates to subsequent development and achievement, but that this relationship is mediated by family background and environment and by subsequent life experiences.

A total of 11,000 cases will be drawn from the TALENT sample. Of these, 2000 will be only children, with 1000 children each from eight other birth status postions (e.g., oldest and youngest in a 2-child family, eldest, second, and youngest in a 3-child family, eldest second, other intermediate, and last in a family of more than 3 children). Variables to be investigated include parents' characteristics; family environment; outcomes and experiences in high school such as grades, cognitive skills and achievement; and outcomes and experiences at age 29 such as life quality and achievement.

Significance to Biomedical Research and the Program of the Institute: This study is relevant to the Institute's interests in the consequences of fertility.

Proposed Course: This study is expected to continue into FY 80.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Computer File of Studies on the Psychosocial Antecedents of Adolescent Pregnancy and Childbearing 1970-1980
Contract Number : NO1-HD-92800
Contractor : Western Washington University
Money Allocated : $122,509 (1979)

Objectives: The purpose of the study is to construct a computer file of major studies of psychological and social antecedents of adolescent pregnancy and childbirth. Nine studies conducted during the 1970s have been identified, however, other studies might be added. A major goal of the project is to produce an easily used data file available for secondary analysis. This will entail obtaining the data from principal investigators of the studies, establishing the quality of the data and the comparability of information from different studies, completing the file construction, testing the file, and writing a user's codebook and manual. Studies included in the file will be those which have measured psychological or social variables, have collected data on a large sample of individuals between the ages of 13 and 19 of either, or both, sexes, and meet accepted standards of scientific reporting of research methods and sampling.

Significance to Biomedical Research and Program of the Institute: This project will make available to the scientific community a large data set containing information on an important biomedical and social topic--adolescent pregnancy and childbearing. Early childbearing has been shown to affect both the health and the social well-being of the mother and child through factors such as low birthweight, pregnancy complications, mother's education, and socioeconomic status. The collection of data is both costly and time consuming and frequently the data are analyzed only by the original investigator. Further analysis of previously collected data can aid in answering many of the questions in the area of adolescent pregnancy and childbearing.

Major Findings: Preliminary work has found a substantial degree of equivalence from one study to another in the measured variables. Up to one quarter of the variables are equivalent across all studies and an even greater number are common to two or more studies. Thus, a working file of data from a number of studies can be created.

Proposed Course: This project was begun in Fiscal Year 1979.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : The Intellectual, Physical, Psychological and Social Consequences of Being Reared an Only Child
Contract Number: NO1-HD-9-2802
Contractor : Emory University
Money Allocated: $69,938 (1979)

Objectives: This study is undertaking cross-sectional and longitudinal analyses of Cycles II (1964-65) and III (1966-70) of the National Health Examination Survey data on the only child compared with children in multiple-child families in order to identify differences in characteristics between only children and others and to see whether such differences can be accounted for by the mediating and control variables such as parents' education and child rearing practices. Variables include: consequences--physical condition, social and psychological adjustment, and intellectual development; parental child-rearing practices; and other mediating control variables such as parents' education, etc.

Major Findings: Preliminary findings indicate that cognitive development of only children, as measured by performance on a variety of tasks, generally is more advanced compared to that of children with siblings, particularly middle-born children. Physical development, measured by the percent of children who walked before age one, appears to be faster for only-and eldest children. Medical records show five percent more of the only children had something wrong with them as a baby, but the only child and the eldest child were significantly healthier at the time of examination than were the middle-and the youngest born. The belief that only children have psychological adjustment problems to a greater degree than non-only children is not born out by the data on temperament, attention span, aggressiveness, meeting new children, or making new friends. Only children did not differ from others with respect to number of friends, participation in group activities, and leadership. Only- and eldest children were rated as more "tense and high strung," but a significantly greater number of middle-born children were recommended for help for "being emotionally disturbed," and more had "talking problems."

Significance to Biomedical Research and Program of the Institute: This research, requested under RFP78-9 relating to "childlessness and the one-child family," is important to the biomedical and behavioral programs of the Center and is policy relevant to departmental and Congressional concerns. Consequences addressed in this study include variables close to the concerns people have about only children.

Proposed Course: This project, initiated in FY 79, is scheduled to be completed in FY 81.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : The Effects of Childlessness on Life Satisfaction in Old Age
Contract Number : NO1-HD-9-2803
Contractor : International Fertility Research Program
Money Allocated : $59,546 (Est. 1979)

Objectives: This study will analyze longitudinal data collected by Duke University in the Durham, North Carolina area to determine the consequences of being childless in old age (or of having children living at some distance) in terms of life satisfaction, health status, patterns of leisure time and financial resources for support. The Duke University Longitudinal Study of Aging began in 1955 with 256 male and female respondents over age 60, and the Duke University Adaptation Study began in 1968 with 502 respondents aged 45-69. Longitudinal comparisons are to be made of relatively age-homogeneous populations of elderly with children vs. those who are childless. Analysis will also differentiate among the "functionally childless" (those who have children but who are relatively isolated from them by distance) and the voluntary vs. involuntary childless; differences in earlier economic and occupational status will be analyzed for each group so as to compare with later psychological well-being and health. The study will also see whether childless old people compensate for the lack of children by utilizing substituttes, or functional alternatives, for children such as other relatives, neighbors, friends or institutional facilities.

Significance to Biomedical Research and Program of the Institute: Little research is available on the relationships to be investigated. This research, requested under RFP 78-9 relating to "childlessness and the one-child family", is very important to the biomedical and behavioral programs of the Center and is highly policy relevant to departmental and Congressional concerns. Policy implications focus on the consequences of childlessness for the health and well-being of the elderly and, thus, on whether "additional resources will need to be allocated to institutional facilities and programs aimed at caring for the aged as a result of the aging of the population and an increase in childlessness and one-child families in the United States."

Proposed Course: This project is scheduled to get under way in FY 79 and to be completed in FY 80.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Childlessness and Status Attainment
Contract No. : NO1-HD-92804
Contractor : University of Texas
Money Allocated: $40,974 (1979)

Objectives: The aims of this study divide into three groups: (1) the detailed demographic description of childlessness from 1910 to the present by cohort; (2) the development and implementation of a measurement strategy for separating the phenomenon of childlessness into voluntary and involuntary categories, and an attempt to see how these two categories have changed over time; (3) the assessment of the impact of voluntary childlessness on wives' status attainment variables such as family earnings and occupational prestige, and the comparison of the status attainment effects of voluntary vs. involuntary childlessness.

The first part of the study utilizes Census and CPS data and the last two parts, cross-sectional data sets from 1955, 1960, 1965, 1970, and 1973 national fertility studies.

Significance to Biomedical Research and Program of the Institute: This research, requested under RFP 78-9 relating to "childlessness and the one-child family," is important to the biomedical and behavioral programs of the Center and is policy relevant to departmental and Congressional concerns. It provides demographic descriptive data on childlessness and addresses the consequences of childlessness for individuals and families.

Proposed Course: This project is scheduled to get underway in FY 1979 and to be completed in FY 1980.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : A Study of the Consequences of Deciding to Remain Childfree, Compared with Deciding to Have Children and Deciding to Postpone Children.
Contract Number : NO1-HD-92805
Contractor : University of North Carolina
Money Allocated : $71,425 (1979)

Objectives: This project is designed to analyze six key attitudinal, lifestyle, achievement and economic consequences of remaining voluntarily childless. The voluntarily childless shall be compared with postponers and parents. Voluntarily childless wives will be compared with other wives to test the hypothesis that the voluntarily childless wives will be more likely to: (a) emphasize the costs of children, negative motivations for parents having children and positive motives for remaining childless, as well as the negative consequences for children of parenthood; (b) be characterized by dyadic withdrawal including avoidance of participation in formal and informal organizations and clubs and by emphasis on solitary couple activities; (c) have an egalitarian division of labor in the home; (d) have consistently high achievement for the wives, and perhaps somewhat lower achievement for the husbands; (e) have high marital satisfaction; (f) have higher combined income, less debt, and higher satisfaction with their standard of living. The study will also investigate whether voluntary childlessness is a qualitatively unique phenomenon or a quantitative extension of a model that explains low parity processes. Analyses will be performed on extant data from a random sample of 186 voluntarily childless and 598 other married women.

Significance to Biomedical Research and Program of the Institute: This study is relevant to the Institute's interests in the antecedents, determinants and consequences of fertility.

Proposed Course: This study is expected to continue into FY 80.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Differential Consequences in Early Adult Life of Having Been an Only Versus a Sibling Child
Contract Number : NO1-HD-9-2806
Contractor : Bowling Green State University
Money Allocated : $68,803 (1979)

Objectives: Using extant data from the 1973 National Center for Health Statistics, National Survey of Family Growth, the investigators propose to investigate consequences in later life of having been an only child. Using the Family Growth Survey national probability sample of women ages 15-44, the researchers will investigate the differential consequences of "only" versus "sibling" children on educational attainment, family formation and labor force participation. Women from sibling families who are currently married to men who were only children will also be examined if sufficient data are available.

The researchers will view the results within a developmental framework to determine at what stages within the first half of the adult life cycle differential social and economic outcomes for only children may be different from those for sibling children. The overall study hypothesis is that women who were only children, or who have married men who were only children, will be more likely than sibling children to achieve selected patterns of educational attainment, family formation, and labor force participation.

Significance to Biomedical Research and Program of the Institute: This research is relevant to the Institute's interests in the consequences of fertility.

Proposed Course: This project will continue into FY 80.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Long Term Consequences for Couples of Childlessness and One Child Families
Contract No. : NO1-HD-9-2807
Contractor : The Pennsylvania State University, University Park, Pennsylvania 16802
Money Allocated : $59,812 (1979)

Objectives: This study will utilize a 24-year longitudinal study of married women in Pennsylvania to systematically evaluate the consequences for parents of being childless or of having only one-child and also to examine several antecedent factors. Some 915 women from rural backgrounds, married only once and still living with their husbands, were interviewed as high school sophmores in 1946 (at about age 16), in 1957, and again in 1971, so they were about 40 years of age at the last interview. The data set, which includes 45 childless women and 75 currently married women with one child, will allow an examination of the consequences for parents of childlessness and the one-child family. Antecedent factors such as personality, family of orientation and birth order, occupational aspirations, etc., are available from the early data set. Consequences of occupational and income aspirations and achievement, life satisfactions, drive for social mobility, and social participation are available in the last data set. Comparisons and causal models can be produced from this extant longitudinal data set.

Significance to Biomedical Research and Program of the Institute: This research, requested under RFP 78-9 relating to "childlessness and the one-child family," is very important to the biomedical and behavioral programs of the Center and is highly policy relevant to departmental and Congressional concerns.

Proposed Course: This project is scheduled to get underway in FY 1979 and to be completed in FY 1981.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Sexual and Contraceptive Attitudes in Behavior of Young, Single Females
Contract Number : NO1-HD-92809
Contractor : The University of Guelph
Money Allocated : $26,354 (1979)

Objectives: This project will analyze two data sets relating to adolescent sexuality and contraception. Both data sets were collected in Ontario, Canada and involve data from a film evaluation study of 447 females aged 17 to 22 and from a birth control clinic study of 486 females aged 13 to 20. The research will focus on sexual abstinence, type pologies of abstinence, measurement of contraceptive use, predictors of contraceptive use, contraceptive embarrassment, and relationship between abortion attitudes and contraceptive behavior.

Significance to Biomedical Research and Program of the Institute: Teenage pregnancy is a institute program of high priority. This project is directly relevant to this program and is regarded as a promising research on teenage sexual and contraceptive behavior. The project relates to the antecedents of teenage pregnancy and its findings should be very relevant to the prevention of teenage pregnancy.

Proposed Course: This project was funded in Fiscal Year 1979 and will be completed during Fiscal Year 1980.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Research on Childlessness and the One-Child Family
Contract Number : NO1-HD-92814
Contractor : Steiger, Fink and Kosecoff Incorporated
Money Allocated : $39,719 (1979)

Objectives: This project will examine the social, psychological, and physical consequences of family size among couples who have completed or almost completed their childbearing. Special emphasis will be on couples who have zero or one parity. The data come from the Rand Corporation's Health Insurance Study which has involved 8000 individuals in 2,700 families in four geographic locations in the United States. Some limited additional data will be collected by the Rand Corporation. The consequences to be investigated will include social adjustment, social networks, career patterns, income, psychological well-being, perceived quality of life, current health status, resistence/susceptibility to illness and concern for health.

Significance to Biomedical Research and Program of the Institute: Fertility and family size are major areas of concern within CPR. The consequences of childbearing relate directly to future decisions concerning fertility and family size. This project will investigate the special cases of families with no children or only one child. It is anticipated that the research findings will be of significant importance to public policy makers and to individual families.

Proposed Course: This project was begun in Fiscal Year 1979 and will terminate in Fiscal Year 1981.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : The Disruptive Effects of Fertility: A Longitudinal Study of the Sequential Consequences of Childlessness and Childbearing on the Educational and Occupational Pursuits of College-educated Women
Contract No. : NO1-HD-9-2816
Contractor : DePaul University
Money Allocated: $42,098 (1979)

Objectives: This is a project to study consequences of childbearing of one and more than one child and childlessness on such variables as education/occupational aspirations and achievements, toward a goal of developing a parsimonious model, which focuses on: initial aspirations, fertility/career choices, and the relationships between these. The contractor will analyze extant data tapes containing longitudinal information collected by NORC on 1961 college graduates, collected in 1961 on a representative sample (N=41,116), with a subsample of 4868 completing five waves of the survey (in 1961, 62, 63, 64, and 1968), and using a subsample of 1685 women college graduates for the analysis of short term consequences of childbearing of one and more than one child and childlessness.

Significance to Biomedical Research and Program of the Institute: This study is relevant to the Institute's interests in the consequences of fertility.

Proposed Course This study began in FY 79 and is expected to continue into FY 81.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Study of Consequences of Remaining Childless or Having Only One Child
Proposal No. : 78-9-037
Contractor : Research Triangle Institute, Research Triangle Park, N. C.
Money Allocated: $235,891 (Est. 1979)

Objectives: This study will (a) analyze the 1973 National Survey of Family Growth and the 1971 and 1975 Current Population Surveys and (b) hold discussions with 60 couples to assess the long-term social, economic and social-psychological consequences of remaining childless or having only one child for couples; experienced quality of life, especially during mid-life (ages 35-54), covering part of the childrearing years and entry into the "post-parental stage" of life, as well as a major segment of worklife.

The investigators will study the consequences of childlessness and of having only one child rather than 2 or more by examining the adult life experience, focusing on important economic, demographic and social-psychological aspects of the quality of life experience, i.e., (a) marital dissolution and remarriage; (b) women's labor force participation and occupational attainment; (c) family income and standard of living; and (d) the degree of congruence between women's educational and occupational attainments, and between spouses' occupational attainments.

They will also examine in depth the ways in which couples experience the social, economic and psychological "costs" and "benefits" of remaining childless or of having one child or several children.

The analyses of large-scale studies of the "objective" aspects of quality of life will be combined with in-depth studies of couples' experiences in these areas and in marital and parental roles, friendships, and use of financial resources and leisure time.

Significance to Biomedical Research and Program of the Institute: This research, requested under RFP 78-9 relating to "childlessness and the one-child family," is important to the biomedical and behavioral programs of the Center and is policy relevant to departmental and Congressional concerns.

Proposed Course: This project is scheduled to get underway in FY 1979 and to be completed in FY 1981.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : The Consequences of Being and Having an Only Child on Intelligence, Interpersonal Orientation, Attitudes and Time Use
Proposal No. : 78-9-053
Contractor : The University of Texas at Austin
Money Allocated: $191,679 (est.)

Objectives: It is proposed to do a series of three interrelated studies to assess the consequences of being and having an only child on intelligence, interpersonal orientation, attitudes and time use, over a two year period. The research is designed to examine the consequences of growing up without siblings particularly (but not solely) on the development of interpersonal skills and IQ. The research studies are grounded on the confluence model of intellectual development proposed by Zajonc and Markus which accounts for the relationship between family size and intelligence by explaining the IQ discontinuity of only children as caused by the fact that only children do not have younger siblings to tutor.

Study 1 involving 144 families, 48 of which are one-child families, and 96 two-child families, will examine and compare the activities of family members, and the intellectual abilities and personalities of the children. The study is expected to provide descriptive data about one- and two-child families. Study 2 is designed primarily to examine the effects of teaching someone younger on intellectual abilities of the teacher. Older subjects, 15- and 16-year-olds, will teach younger subjects, 14- and 15-year-olds. Only children will be compared with first and last borns from two-child families. Because the predicted differences do not reliably appear until adolescence, the subjects for these studies will be that age group. Study 3 will involve 360 female undergraduates and is primarily designed to assess the cumulative effects of repeatedly teaching someone younger on intellectual abilities.

Significance to Biomedical Research and Program of the Institute: This study is relevant to the Institute's interests in the antecedents, determinants and consequences of fertility.

Proposed Course: This study is expected to continue into FY 81.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Reciprocal Interaction Skills and Ego Identity Formation in Adolescents from One- and Two-child Families: An Investigation of Family Structure and Family Process Antecedents
Proposal No. : 78-9-054
Contractor : The University of Texas at Austin
Money Allocated: $177,888 (est.)

Objectives: The study proposes to analyse data on 40 only children (half of each sex), 80 first children and 80 second children of two-child families. Half of the second and third children will have siblings of the same sex and half will have siblings of the opposite sex. Three clusters of variables believed to mediate the consequences of being an only child will be examined. These are: Parental attitudes concerning family size and achievement; sibling and peer interaction; and family interaction and climate. Because only children may have different experiences than children with siblings relative to these three factors, it is possible that they will exhibit distinctive patterns of ego identity and reciprocal interaction skills. The investigator will also study the relationship of process and status to each other and their joint effects.

Significance to Biomedical Research and Program of the Institute: This study is relevant to the Institute's interests in the antecedents, determinants and consequences of fertility.

Proposed Course: This study is expected to continue into FY 81.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : "Sex Role Development and the Single Child Family"
Proposal Number: 78-9-087
Contractor : Institute for Research on Social Problems
Money Allocated: $158,986 (est.)

Objectives: The major objective of the proposed research is to study sex-role development in only children, together with its parental antecedents. Attention will be given to the consequences of being an only child for sex-role development and identity. Also, the effects of having an only child on the parents' family planning decisions and attitudes toward sex-roles will be considered. The research will explore the strength of the parents' need for child gender diversity, the relation of this to family size decisions, and the parents' sex-role expectations in relation to family planning decisions.

The investigator will first compare the parents of only children with the parents of two-child families in such characteristics as sex-role attitudes, degree of sex-typing, perceptions of their child's sex-role orientation, and factors related to family size decisions. Secondly, the study will compare the sex-role identity of the only children with both the first-and last-born children at the pre-school (ages 4-5) and fourth grade (ages 9-10) levels.

Significance to Biomedical Research and Program of the Institute: This research is relevant to the Institute's interest in research concerned with the social, psychological, and economic consequences of childlessness or of having one child.

Proposed Course: Preliminary clearance was received in FY 79 and the work is to begin September 1, 1979, unless delayed.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Center for Population Research
Reproductive Sciences Branch

Within the context of the mission of the Center for Population Research (CPR), NICHD, the Reproductive Sciences Branch (RSB) facilitates the acquisition and application of the knowledge required to enable men and women to: 1) control their fertility; and 2) free themselves from reproductive disease and disablement. The primary means by which such facilitation of the CPR mission is accomplished by the RSB is through the award of grants for the support of research and training in the reproductive sciences. During the period from October 1, 1978 to September 30, 1979, the RSB budget of approximately $38,500,000 was used to provide grants and other forms of support for activities subsumed by the following programs: 1) Fundamental Biomedical Research on Problems of Human and Relevant Animal Reproduction; 2) Institutional Programs in Multidisciplinary Reproductive Sciences Research; 3) Reproductive Sciences Research Manpower Development; and 4) Reproductive Sciences Research Facilitation and Augmentation.

1. Fundamental Biomedical Research on Problems of Human and Relevant Animal Reproduction

A. Fertility-Infertility

Regulation of Gene Expression by Reproductive Hormones. Significant advances were made in studies using the estrogen-ovalbumin system of the chicken as a model for hormonal regulation of gene expression. During the past year, the complete molecular organization of the DNA sequences composing the ovalbumin gene was reported. The direct analysis of DNA sequence organization confirms the gross level structure reported last year, but also provides significant new details of the internal structure of the gene. This latter aspect has raised a great deal of excitement concerning the possible elucidation of the mechanism(s) of gene transcription. It was reported last year that the ovalbumin gene consists of regions termed structural gene sequences and intervening gene sequences. The structural sequences are separated into eight sequentially colinear pieces by seven intervening sequence pieces of various lengths. The lengths of the seven intervening sequence regions have been sequenced and are all located in the region of the gene identified for peptide coding. The determination of possible sequence homologies at the structural-intervening sequence junctions thus became of major importance in view of the hypothesis that intervening sequences must be excised from a large, precursor RNA to form a smaller, functional ovalbumin messenger RNA. The predicted base sequence redundancy has been found at all junctions contained within the hexanucleotide (5')T-C-A-G-G-T(3'). The sequence C-A-G-G, termed the "consensus" sequence, appeared in every junction sequence with an exact splice point at either the A-G or G-G position. This "linking sequence" appeared not only in the 12 splice points of the chicken ovalbumin gene, but also in the immunoglobin and

globin genes of mice. Its evolutionary conservation provides strong evidence for the general importance to transcription of the "consensus" sequence organization within the gene. A molecular mechanism for transcription induced by steroid hormones can now be envisioned which is more complete than ever before. Steroid hormone regulated transcription of both the structural and intervening sequences occurs at similar rates in a coordinated fashion. The sequence of about 8000 bases of DNA used during transcription appears to produce a large (40S), high molecular weight precursor molecule to ovalbumin messenger RNA which undergoes a process of intervening sequence excision with appropriate structural sequence pieces being joined together to form a continuous, functional messenger RNA consisting of only 1859 bases.

Molecular Basis for Gene Malfunction. Demonstration of a molecular basis for detecting genotypic alleles and description of their molecular organization has been reported. Using recombinant DNA technology and restriction enzyme mapping of intragene DNA organization, two allelic forms of the natural ovalbumin gene of the chicken have been independently cloned and characterized. They are different with regard to a single Eco RI cleavage site in one of seven intervening sequences within the gene. This difference, based on DNA sequence analysis, may be created or eliminated by a single base mutation within the intervening sequence of the ovalbumin gene. Individual chickens of the same breed are 10% homozygous with an extra Eco RI cleavage site, 40% homozygous without such a site and 50% heterozygotes. The possible existence of a series of such mutational variations within the ovalbumin gene has been shown by similar studies using a second restriction enzyme (Hae II) which demonstrated two cleavage sites in some, but not all of the individual chickens studied. Thus, there appear to be several additional allelic variations possible within the ovalbumin gene, one of which represents a change within an intervening sequence. This latter type of mutation within a eukaryotic gene has no known phenotypic manifestation and represents an entirely new type of silent mutation. Work of this nature demonstrates the feasibility of mapping the fine-structure of gene variations associated with reproductive processes and their possible disorders and may serve as the basis for the future development of molecular medicine.

Androgen Binding Protein. Good progress continues to be made in the exciting new area to establish links between the biochemical actions of androgens and the gonadotropin FSH. Such studies may also establish fundamental principles applicable to steroid-peptide hormone actions on other cell types. Androgen binding protein (ABP) is the first secretory product of the Sertoli cell of the testis to have been isolated to homogeneity and thus be available for detailed studies. Recently, several laboratories have demonstrated that this testicular protein is secreted also into the blood. The preparation of antibodies to the purified protein has led to the development of a radioimmunoassay for ABP. The complex hormonal regulation of ABP continues to be elucidated. It now appears that the apparent LH stimulation of ABP in vivo was due to the increase in intratesticular testosterone which stabilized ABP activity and

thus increased the recovery of ABP. Treatment with anti-LH only partially reduced stimulation of intratesticular testosterone in response to FSH but nearly abolished the response to LH. Since LH increases intratesticular testosterone but does not increase ABP in vivo, it seems unlikely that testosterone mediates the acute effects of FSH on ABP even though FSH stimulation of ABP is associated with an increase in intratesticular testosterone. Therefore, it is concluded that FSH stimulation of ABP and intratesticular testosterone is independent of endogenous or exogenous LH.

Sperm Maturation. In the past few years, much progress has been made in the acquisition of new basic knowledge of male reproductive processes. The development of better techniques to prepare relatively pure cell types from the testis and epididymis has allowed investigators to make progress in characterizing the biochemical milieu of the developing sperm. Substrate and enzyme levels in cell types derived from different parts of the epididymis can be examined with relative accuracy and correlations attempted with sperm maturation. (Sperm mature and acquire the ability to be mobile and to fertilize as they traverse the epididymis and are exposed to epididymal secretions.) It is known that epididymal plasma contains carnitine and glycerol phosphorylcholin (GPC) in high concentrations. High levels of GPC have now been found in rat caput epididymal cells. It also appears from investigations of factors which inhibit the initiation of sperm motility in the epididymis that carnitine and especially GPC may have the biologically important function of keeping sperm quiescent during storage, and thus, preserving energy for the time of ejaculation.

Gonadotropin Biosynthesis. Progress continues to be made in characterizing the mechanisms of biosynthesis of the pituitary glycoprotein hormones. For the first time evidence has been presented for the cell free synthesis of pituitary luteinizing hormone subunits alpha and beta. The in vitro translation of bovine pituitary RNA was used to examine this biosynthesis. Results suggest that the alpha subunits of the pituitary glycoprotein hormones are synthesized in precursor form and that the subunits are synthesized separately, as has been shown for hCG and TSH. Studies of the structure of human follicle stimulating hormone have progressed to the point where they can provide a preliminary synthetic approach to glycoprotein hormones. Data suggest that the alpha subunit lies on the surface of the native hormone.

Biorhythms and Receptors. An interesting observation recently made is that of seasonal variation in progesterone receptors in the oviducts of estrogen treated immature chicks. The receptor displays seasonal variations in levels, molecular species, nuclear binding capacity, and effects on RNA polymerase activities. The progesterone receptor is considered to be a dimer consisting of an A and B monomer species, each bound with one progesterone molecule. In the winter the progesterone receptor loses its capacity to bind nuclear acceptor sites both in vivo and in vitro. This is accompanied by a corresponding loss in one receptor species, A. In the latter half of the year when this receptor is high, receptor binding appears

to be normal.

B. Fertility Regulation

LRF Analogs. Luteinizing hormone releasing factor (LRF) is a decapeptide which stimulates the secretion of pituitary gonadotropins necessary for normal reproductive functions. Analogs have been prepared which either mimic or inhibit the actions of native LRF. These compounds are being actively investigated for their diagnostic and therapeutic potential. In the past year several laboratories have shown that intermittent administration of low doses of LRF agonists results in clinical improvement in patients with reproductive dysfunctions, e.g., hypogonadotropic hypogonadal males and amenorrheic females whose symptoms are secondary to hypogonadal dysfunction. The dosage and the timing of administration of LRF compounds appear to be critical for the type of response elicited. When higher doses were administered, the improvements in gonadal function were reversed. Consideration of these data suggest that the antireproductive effects of LRF analogs can be exhibited in men and women.

Extrapituitary Action of Gonadotropin Releasing Hormone (LRF). There is exciting new information on the role of this important neuropeptide. A number of laboratories have reported data which indicate that several peripheral organs contain receptors to the brain neuropeptide LRF. Preliminary data appear to demonstrate that LRF, in addition to its well-known gonadotropin releasing action in the pituitary, also exerts a direct effect on ovarian and testicular function. These data offer new areas to explore for possible application to fertility regulation.

Implantation. Implantation initiates complex growth processes in the uterus. Uterine endometrial cells may release hydrolytic lysosomal enzymes when their functions are redirected by hormones. Cellular breakdown occurs as the uterus is invaded by the fertilized ovum and this process has been investigated for possible lysosomal involvement. The lysosomal cathepsin D enzyme was purified and an antibody prepared against it. Immunohistochemical localization of cathepsin D shows a significant decrease in concentration in implantation sites as compared to interimplantation sites. These histological observations are supported by previous biochemical data. Investigations of the mechanisms by which estrogen and progesterone control the synthesis and degradation of lysosomal enzymes add to our fundamental knowledge of the process of implantation, its control, and its possible inhibition.

C. Nutrition and Reproduction

Oral Contraceptives and Pyridoxine (B6). Much uncertainty still exists regarding the impact of oral contraceptives on B6 metabolism. It appears that estrogen-based oral contraceptives increase the requirements for vitamin B6 in the same way that occurs during pregnancy. Preliminary data from a CPR supported laboratory suggest that long term use (over 30 months) of oral

contraceptives prior to conception resulted in poor vitamin B6 nutrition in both mother and child. Thus, it would appear that the increased needs for vitamin B6 associated with pregnancy are further increased when pregnancy follows long-term use of oral contraceptives. Whether or not these differences are injurious to mother or child cannot be answered at present.

Prostate and Zinc. The prostate appears to be unique in its function of accumulating and secreting high concentrations of citrate under the regulation of androgen. In studies to determine the biochemical reasons for this high level of citrate, a grantee has obtained preliminary data to suggest a role for zinc. It should be noted that the prostate normally has high levels of zinc as well as of citrate. Limited activity of the enzyme aconitase has been shown to be a major factor in the low citrate oxidation characteristic of prostate mitochondria. Zinc now appears to be a strong inhibitor of aconitase activity. Thus, it would seem that zinc has an important role as a requirement for high citrate accumulation by inhibiting aconitase in the prostate.

D. Reproductive Diseases

Diethylstilbestrol (DES). The development of the guinea pig as an animal model system analogous to that of the human male has been successfully accomplished by a CPR grantee to study the effects of prenatal administration of estrogen to the pregnant mother on male sexual development. This model provides opportunities for new insight into the mechanisms of changes reported in the human male offspring of women exposed at critical periods of pregnancy to DES: defects in sperm production, possible fertility problems, metaplastic changes in accessory duct epithelium with the possibility of malignant changes in later life. Another investigator is assessing the prevalence of upper reproductive tract anomalies and urinary tract anomalies in a patient population at risk for DES induced anomalies of the cervix and vagina. While the medical community has a good understanding of the effects of prenatal DES on the lower genital tract of women, a serious question exists about the prevalence of upper reproductive tract anomalies in this group and, thus, an elevated risk of unsatisfactory pregnancy outcome. Preliminary data appear to indicate that pregnancy wastage may be higher in the DES exposed offspring with normal uteri. This is a timely study as the majority of these women are in their prime childbearing years now.

Mycoplasmosis. The growing belief that mycoplasmal infections may contribute to human infertility has been difficult to assess for the evidence has been only epidemiological. The establishment of a rat animal model now allows a systematic investigation of the specific mechanisms involved in the induction of infertility by mycoplasma-host interactions. The slow development of lesions in the rat suggests that genital mycoplasmosis in the human being might also be slow evolving, thus making its effects upon reproduction much more subtle. Preliminary data from infected pregnant rats show a 50% fetal loss.

2. Institutional Programs in Multidisciplinary Reproductive Sciences Research

The RSB supports three types of Institutional Programs in Reproductive Sciences Research: Program Projects, Specialized Reproductive Sciences Research Centers, and Reproductive Sciences Research Centers at leading institutions in the United States. Support for these programs is provided to enable such institutions to develop and to conduct reproductive sciences research programs involving such disciplines and such critical masses of highly skilled scientists as may be required for attacks on complex problems in the reproductive sciences that are beyond solution by the single investigator working alone or in relative isolation in his discipline. Twenty Institutional Programs were supported during the period from October 1, 1978 to September 30, 1979.

A. Program Projects

The recipient institutions and principal research areas of the Program Projects supported during the period from October 1978 to October 1979 are: 1) Columbia University - reproductive biochemistry relevant to problems of human reproduction; 2) The Oregon Regional Primate Research Center - factors which control fertilization in nonhuman primates; 3) The University of Pennsylvania - biochemical and biological events in reproductive processes prior to implantation; 4) Harvard University - reproductive biology and fertilization; 5) University of California at San Diego - gametogenesis and reproductive biology; and 6) University of Texas at Dallas - neuroendocrine and behavioral interrelationships.

B. Reproductive Sciences Centers

The recipient institutions and principal research areas of Reproductive Sciences Research Centers supported during the same period are: 1) Vanderbilt University - reproductive physiology and endocrinology; 2) Baylor College of Medicine - mechanism of hormone action; 3) Columbia University - steroid biochemistry and female reproduction; 4) University of Pittsburgh - primate reproduction; 5) Harvard University - reproductive biology and human reproduction; and 6) University of Texas at San Antonio - reproductive mechanisms.

C. Specialized Reproductive Sciences Research Centers

The recipient institution and principal research areas of the Specialized Reproductive Sciences Research Centers supported during the same period are: 1) The University of Michigan - reproductive endocrinology from molecular to physiological levels; 2) The University of Texas at Austin - control mechanisms at molecular to organismic levels; 3) Case Western Reserve University - reproductive biology of events from ovulation to implantation; 4) University of Texas at Houston - male reproductive function; 5) Washington University - hormonal regulation of the reproductive tract; 6) University of Washington - gamete transport in mammals; 7) Mayo Foundation - mechanism of action and molecular biology of reproductive hormones; and 8) Salk Institute for Biological Studies - neuroendocrinology of reproduction.

3. Reproductive Sciences Manpower Development

A. Institutional Fellowships

The Institute awards grants for maintenance of institutional environments for research training in the reproductive sciences and for the support of both predoctoral and postdoctoral trainees selected for study in these environments. During fiscal year 1979, NICHD supported 25 reproductive sciences research training grants to 23 institutions. A total of approximately $1,829,000 was used in 1979 by the institutional recipients of research training grants for the support of 118 trainees. Of these trainees, 56 were predoctoral and 62 were postdoctoral fellows. During this year, 6 training programs authorized under the previous training act were phased out.

B. Individual Postdoctoral Fellowships

In addition to institutional grants for research training, NICHD awards postdoctoral fellowships to individuals for the support of their training in research in the reproductive sciences. During fiscal year 1979, a total of approximately $1,122,000 was awarded to 70 postdoctoral scientists.

C. Research Career Programs

The Institute awards Research Career Development Awards (RCDA's) to outstanding senior postdoctoral candidates to enable them to devote full-time to the development of expertise in research in the reproductive sciences. These awards provide up to $30,000 for salary to recipients for a maximum of five years of support. CPR supports two Research Career Awards (RCA's) to established investigators. Approximately $636,260 was spent in fiscal year 1979 for the support of 17 RCDA's and 2 RCA's.

4. Reproductive Sciences Research Facilitation and Augmentation

A. Conferences, Workshops, and Symposia

Conferences, workshops, and symposia are sponsored by RSB, CPR to facilitate the exploitation of new knowledge in critical areas of research in the reproductive sciences and to facilitate the rapid dissemination of new and significant information regarding the reproductive sciences. The following meetings were sponsored during the period from October 1, 1978 to September 30, 1979.

The Annual Meeting of Directors of CPR-supported Population Research Centers and Program Projects was held in Philadelphia, Pennsylvania, May 1979 and it was hosted by Dr. Luigi Mastroianni, University of Pennsylvania.

A symposium on Developmental Reproductive Biology and Sexual Differentiation was held in Quebec City, Canada, August 1979. It was sponsored by the Society for the Study of Reproduction.

An International Conference on Cellular and Molecular Aspects of Implantation was held in Houston, Texas, September 1979, sponsored by the Baylor College

of Medicine and CPR. Organizers were Drs. S. R. Glasser, D. W. Bullock, and W. A. Sadler.

A Workshop on Future Goals of Male Reproductive Medicine and Surgery was held in Bethesda, Maryland, September 1979, and was sponsored by NICHD. Drs. Boyarsky and K. Polakoski of Washington University School of Medicine were the organizers.

CENTER FOR POPULATION RESEARCH

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8-2812	Synthesis and Testing of Progesterone Analogues of Novel Structures with Potential Antifertility Activities	
8-2814	Specific Immunization Against FSH as a Male Contraceptive	
8-2815	Synthesis and Testing of Novel Steroids with Potential Male Contraceptive Activity	
8-2816	Synthesis and Testing of Male Contraceptive Agents	
8-2817	Synthesis and Testing of Male Contraceptive Agents	
8-2819	Synthesis and Testing of Analogs of LHRH as Ovulation Inhibitors	
8-2820	Peptide Antagonists of LHRH as Ovulation Inhibitors	
8-2821	Peptide Antagonists of LRF as Ovulation Inhibitors	
8-2822	Peptide Antagonists of LHRH as Ovulation Inhibitors	
8-2823	Synthetic Chemical Facility	
8-2825	Analysis of Data from the Study of Correlation of Cervical Mucus Changes with Serum Hormone Levels	
8-2830	Pharmacokinetics of Testosterone Enanthate in Human Male	
8-2856	Development and Testing of Vaginal Contraceptives	
8-2857	Clinical Investigation of a Polyurethane Contraceptive Vaginal Sponge Containing a Spermicidal Agent	
------	Development and Testing of Vaginal Contraceptives	

Contraceptive Evaluation Branch

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Steroid Contraceptives--Cancer and Other Neoplasia		
2-2725	Contraceptive Steriods in Beagles	
2-2729	Central Repository for Tissue Evaluation of Contraceptive Steroids	
7-2834	Secondary Amenorrhea, Pituitary Adenoma and Oral Contraceptives	
9-2810	" " " " " "	
9-2811	" " " " " "	
9-2812	" " " " " "	
8-1037	Cancer and Steroid Hormones	
8-2803	Case-Control Study of the Relative Risk of Malignant Melanoma of the Skin Among Women on Contraceptive Steroids	
8-2804	Case-Control Study of the Relative Risk of Malignant Melonoma of the Skin Among Women on Contraceptive Steroids	
9-2813	Decreased Risk of Breast Disease Among Oral Contraceptive Users: Histopathologic Evaluation of Epithelial Atypia	
Steroid Contraceptive--Heart Disease, Hypertension,. and Thromboembolic Disease		
5-2830	Effects of Contraceptive Steroids on Blood Pressure	
5-2831	Effect of Contraceptive Steroids on Blood Pressure	
5-2832	Effects of Contraceptive Steroids on Blood Pressure	
6-2808	Deaths from Myocardial Infarction and the Role of Oral Contraceptives	
6-2849	Case-Control Study of Myocardial Infarction in Young Women with Special Reference to the Role of Oral Contraceptives	
7-2804	The Effect of Oral Contaceptives on Idiopathic Venous Thrombosis: A Study of Antithrombin III and Fibrinolysis	
Metabolic and Nutritional Disorders		
4-2862	Effects of Oral Contraceptives on Folic Acid Metabolism	
8-2818	Relative Risk of Diabetes in Users of Steroid Contraceptives	
Longitudinal Surveillance		
3-2710	Contraceptive Drug Study	
Birth Defects Associated with Contraceptive Practices		
4-2861	Prospective Study of the Effects of Contraceptive Agents, Related Methods and Procedures on the Frequency of Birth Defects	
6-2800	Maternal Factors in Birth Defects	
8-2829	Long Term Effects on Mothers and Offspring of Diethylstilbestrol Treatment During Pregnancy	

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Pathogenetic Mechanisms		
2-2730	A Comparative Study of Progestin Metabolism and Mechanism of Action	
Vasectomy Projects		
2-2770	Effects of Vasectomy on Testis and Epididymis	
2-2776	Endocrine Changes in Vasectomized Men	
3-2717	Immunopathologic Consequences of Vasectomy	
4-2866	Long-Term Effects of Vasectomy in the Rhesus Monkey	
5-2841	Immunologic Studies of Vasectomy	
5-2842	Immunologic Studies of Vasectomy	
6-2807	Health Status of American Men	
7-2801	" " " " "	
7-2802	" " " " "	
7-2803	" " " " "	
8-2827	Effect of Bilateral Vasectomy on the Progression and Regression of Atherosclerosis in Macaca fascicularis	
Serious Hazards of IUD Use		
5-2852	Survey of Womens' Health and Birth Control Methods	
7-2811	" " " " " " " "	
6-2806	" " " " " " " "	
7-2807	" " " " " " " "	
6-2804	" " " " " " " "	
7-2808	" " " " " " " "	
7-2809	Survey of Womens' Health and Birth Control Methods	
7-2812	" " " " " " " "	
6-2805	" " " " " " " "	
7-2810	" " " " " " " "	
Effects of Induced Abortions on Subsequent Pregnancies		
6-2802	A Prospective Study of the Effects of Induced Abortion on Subsequent Reproductive Function	
6-2803	Prospective Study of the Outcome of Pregnancy Following Induced Abortion	
7-2801	Effects of Induced Abortion on Subsequent Reproductive Function and Pregnancy Outcome in Hawaii	
Behavioral Sciences Branch		
1-2138	Support of Bibliographic Preparation of Population Index	
4-2803	The Effect of Birth Order on Mother-Child Relationships	
4-2804	On the Consequences of Reproduction: A Utility Model of Reproductive Behavior	
4-2844	Age at Marriage and Time to First Birth: A Longitudinal Study of Parental Attitudes and Children's Personality	
5-2807	A Study of Couples' Motivations for Parenthood, Decision-Making, and Fertility Regulation	
5-2819	A Longitudinal Study of Intended and Actual Fertility: The 1975 National Fertility Study	

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5-2835	The Impact of Childbearing and Spacing Patterns on the Health of the Mother and the Development of the Child in an Urban Population	
5-2837	An Economic Analysis of the Consequences of Child Spacing and Related Fertility Behavior	
5-2838	Analyses of Relationships Between Fertility, Marriage Dissolution, and Remarriage	
5-2840	Research on the Consequences of Childbearing and Childspacing Patterns for Parents	
3-2799	A Study of Low Fertility Cohorts in the United	
6-2818	States	
2-2707	Development of Technology and Training Program	
6-2828	for Assessing Unit Record Data Files	
6-2829	The Consequences of Early Childbearing: An Analysis of Selected Parental Outcomes Using Two Longitudinal Data Sets	
6-2833	Alienation and Fertility Control in the Early Years of Marriage	
6-2834	Fertility Values and Family Growth	
6-2836	The Social and Demographic Consequences of Teenage Childbearing for Urban Women	
6-2858	Sequelae to Teenage Pregnancy	
6-2859	Research on the Consequences of Adolescent Pregnancy and Childbearing	
6-2860	Effects of Adolescent Pregnancy on Mother-Infant Relationships: A Transactional Model	
7-2805	The Intellectual and Behavioral Status of Children Born to Adolescent Mothers	
7-2806	Economic, Social and Psychological Consequences of Teenage Childbearing	
7-2819	Consequences of Late Adolescent Childbearing in the Early 1970's	
7-2820	Long-Term Consequences for Children of Adolescent Childbearers: An Analysis of Project TALENT Data	
7-2821	Consequences of Teenage Motherhood for Mother, Child and Family in a Black Urban Community	
7-2822	Early Childbearing and Kinship Relations	
7-2823	Relations Between Teenage Pregnancy and Neonatal Behavior	
8-2801	A Continuing Study of Childlessness and One-Child Families	
8-2802	Survey Research on Childlessness & the One-child Family	
8-2806	How Mother's Age and Circumstances Affect Children	
8-2807	The Consequences of Family Structure and Maternal State for Child and Mother's Development	
8-2831	Psychological Factors and Fertility Regulation in Women: Data Analysis	
8-2832	American Attitudes Toward Abortion	

Projects #	Title	Page
8-2833	Psychological Factors Associated with Fertility Regulation: A Comparison of Pregnant and Non-Pregnant Adolescents	
8-2834	A Multifaceted Analysis of Abortion as a Social Issue in the United States, 1965-1978	
8-2835	A Proposal to Investigate the Determinants of Variations in U.S. Fertility Trends 1940-1975	
8-2836	Adolescents' Use of Information about Conception and Contraception	
8-2837	Adolescents' Perspectives on the Health Care Systems: A Determinant of Fertility	
8-2838	Research on the Determinants of Repeated Adolescent Pregnancy and Childbearing	
8-2839	Determinants of Adolescent Sexuality, Contraceptive Use, and Pregnancy Resolution	
8-2840	Sociosexual Development in Black Adolescents within the Family Context: A Longitudinal Study	
8-2841	An Analysis of the Determinants of Adolescent Pregnancy and Childbearing	
8-2842	Contraceptive Use-Effectiveness; The Fit Between User and Method Characteristics	
8-2843	Policy Determinants of Teenage Childbearing	
8-2844	Exploration of the Effects of Organized Family Planning Programs in the United States on Adolescent Fertility	
8-2847	Psychosocial Determinants of Adolescent Contraceptive Use	
8-2848	Determinants of Fertility Behavior Among U.S. Females Aged 15-19, 1971 and 1976	
8-2849	Social and Cognitive Development of Only and First Born Children	
8-2850	The Consequences of Remaining Childless or Having Only One Child: An Analysis of Selected Outcomes for Husbands & Wives	
8-2851	Studies on the One-Child Family	
8-2852	The One-Parent/One Child Family: Sociological and Psychological Consequences	
8-2853	The Personal Meaning of Voluntary and Involuntary Childlessness	
8-2854	The Consequences of Being an Only Child: An Analysis of Project TALENT Data	
9-2800	Computer File of Studies on the Psychosocial Antecedents of Adolescent Pregnancy and Childbearing 1970-1980	
9-2802	The Intellectual, Physical, Psychological and Social Consequences of Being Reared an Only Child	
9-2803	The Effects of Childlessness on Life Satisfaction in Old Age	
9-2804	Childlessness and Status Attainment	
9-2805	A Study of the Consequences of Deciding to Remain Childfree, Compared with Deciding to Have Children and Deciding to Postpone Children	

Project #	Title	Page
9-2806	Differential Consequences in Early Adult Life of Having Been an Only Versus a Sibling Child	
9-2807	Long Term Consequences for Couples of Childlessness and One Child Families	
9-2809	Sexual and Contraceptive Attitudes in Behavior of Young, Single Females	
9-2814	Research on Childlessness and the One-Child Family	
9-2816	The Disruptive Effects of Fertility: A Longitudinal Study of the Sequential Consequences of Childlessness and Childbearing on the Educational and Occupational Pursuits of College-educated Women	
78-9-037	Study of Consequences of Remaining Childless or Having Only One Child	
78-9-053	The Consequences of Being and Having an Only Child on Intelligence, Interpersonal Orientation, Attitudes and Time Use	
78-9-054	Reciprocal Interaction Skills and Ego Identity Formation in Adolescents from One-and Two-Child Families: An Investigation of Family Structure and Family Process Antecedents	
78-9-087	Sex Role Development and the Single Child Family	

NICHD Annual Report
October 1, 1978 through September 30, 1979
Office of the Director, Center for Research
for Mothers and Children

The Center for Research for Mothers and Children (CRMC) supports research and research training activities related to the special health problems of mothers and children in the biomedical, social, and behavioral sciences. Specifically, the CRMC develops and supports basic and clinical research and research training in the biological and behaviroal aspects of, and environmental influences upon, normal and high risk pregnancy, embryonic development, fetal growth and maturation, labor and birth process, postnatal adaptation and maintenance of homeostasis, disorders of infancy, and the effect of perinatal events upon development. The goal is to minimize the risk of morbidity and mortality during the perinatal and infancy period of life.

The CRMC also supports basic research and training for research that is related to the learning process as well as cognitive, personality and social development. Support is given for research that attempts to reveal the nature of the general learning process and certain specific processes such as the acquisition of speech and language and learning to read. The study of specific learning disorders such as developmental dyslexia are also a major concern. Personality, attitude formation and social devlelopment of young children are studied by a wide range of different scientists such as developmental and experimental psychologists, sociologists, anthropologists, and pediatricians. In addition, the Center develops and supports a wide range of biological, behavioral, and social research concerning the causes of mental retardation and related developmental disabilities.

The Center also supports studies on the precursors of adult disease, focusing on congenital defects and developmental immunology, nutrition, metabolism, and endcorinology. The goal is to prevent chronic disabilities by understanding their genetic, nutritional, metabolic and immunologic antecendents.

In December 1978, Dr. Gilbert L. Woodside retired as Deputy Director, NICHD, and Acting Director, CRMC. Dr. Betty Pickett was appointed Deputy Director-Designate, NICHD, in January 1979 (becoming Deputy Director, NICHD in July 1979). She, thereupon, became the Acting Director of the CRMC. A permanent Director of CRMC is being sought by the Institute.

Dr. James F. Kavanagh continued to serve as the Associate Director, CRMC, and, until August, the Acting Chief of the Human Learning and Behavior Branch.

In June 1979, the Pregnancy and Infancy (P&I) Branch and the Developmental Biology and Nutrition (DBN) Branch were combined to form the Clinical Nutrition and Early Development (CNED) Branch with Dr. Eileen Hasselmeyer as the Acting Branch Chief, until July 1979, when she was replaced by Dr. Kavanagh who will serve as Acting Chief until the recruitment of a Chief is completed.

Three sections of this new Branch were established: Genetics and Teratology Section, Dr. Delbert Dayton, Chief; Pregnancy and Perinatology Section,

Dr. Charlotte Catz, Chief; and Nutrition and Endocrinology, Dr. Gilman Grave Chief. For this Annual Report the activities of the recently established CNED Branch were reported under the former Branches, that is P&I and DBN.

Mr. Jehu Hunter, formerly a Health Scientist Administrator and Assistant to the CRMC Director served as a consultant to the Institute with a primary concern for the NICHD Cooperative Epidemiological Study of Sudden Infant Death Risk Factors.

In June, Dr. Hasselmeyer was also appointed Associate Director for Review for the NICHD. Also in June Dr. Phyllis Berman, a developmental psychologist, formerly Associate Professor, joined the Human Learning and Behavior Branch (HLB), as a Health Scientist Administrator.

In August Mr. Philip Sapir, former President of the W.T. Grant Foundation, joined the NICHD as a Special Assistant to the Institute Director for Behavioral Sciences and Mental Retardation and Chief of the HLB Branch.

NICHD Annual Report
October 1, 1978 through September 30, 1979
Pregnancy and Infancy Branch
Center for Research for Mothers and Children

The Pregnancy and Infancy Branch fosters the promotion of a coordinated program of research and research training to enhance advancement of knowledge related to pregnancy and maternal health, embryonic development, fetal growth, and infant well being. Activities evolve around six maternal-infant problem oriented health areas: High Risk Pregnancies; Fetal Pathophysiology; Premature Labor and Birth; Disorders of the Newborn; Congenital Malformations; and the Sudden Infant Death Syndrome.

Program goals recognize the interrelationships of specific health and developmental problems occurring in the prenatal, perinatal, and infant periods of life and the effects that these events may have upon development and well being of the child. Particularly relevant are maternal health problems which affect fetal and infant health, problems with which the newly born infant must cope in adapting to extrauterine life, and events occurring during the neonatal period which can influence the development of the baby.

Branch supported research activities are concerned with the physiology and complications of pregnancy, placental function, immunologic phenomena, lactation, developmental biology, processes of embryonic development and fetal growth, antenatal and intrapartum diagnostic procedures, the process of labor and delivery, and genetic, pharmacologic, toxic, and infectious influences which can affect the pregnant woman and her fetus. Areas of research endeavor related to the newborn infant include respiratory function, metabolic processes and related problems, heat production and temperature regulation, immunology and blood dyscrasias, infectious diseases, congenital malformations, and the sudden infant death syndrome.

The Pregnancy and Infancy Branch is responsible for the administration of approximately 36 percent of the Institute's Center for Research for Mothers and Children extramural holdings, numbering 369 grants and contracts and amounting to approximately 3.4 million dollars. An estimated 8 percent of the Branch's total budget expenditures provide research training support in areas pertinent to pregnancy and maternal health, developmental biology, and perinatology.

An estimated sixty percent of the Branch's research project funds provide support for studies pertaining to High Risk Pregnancy (26%), Fetal Pathophysiology (16%), and Disorders of the Newborn (18%). Nineteen percent (19%) of funds are obligated for the study of Congenital Malformations, fourteen percent (14%) for research specifically on the Sudden Infant Death Syndrome, and eight percent (8%) to studies of Premature Labor and Birth. Program holdings for each area by support mechanism appear in Table I.

NICHD GRANTS AND CONTRACTS, ACTIVE DURING JUNE 1979
Pregnancy and Infancy Branch

Funds in Thousands

Health Area	Total		Total Research						Training Grants (T01)		Fellowships (incl. T32)	
			Total		Grants (incl. RCP's)		Contracts (incl. S06)					
	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds
Total	369	$33,846	323	$31,245	302	$28,645	21	$2,600	9	$977	37	$1,624
High Risk Pregnancy	90	8,879	80	8,616	78	8,316	2	300	2	149	8	114
Fetal Pathophysiology	61	5,283	51	4,809	51	4,809	-	-	-	-	10	475
Premature Labor and Birth	33	2,671	32	2,657	32	2,630	-	27	-	-	1	14
Disorders of the Newborn	78	6,068	66	5,335	58	4,876	8	459	2	237	10	496
Congenital Malformations	70	6,363	57	5,246	57	5,246	-	-	5	590	8	526
Sudden Infant Death Syndrome (Primary)	37	4,583	37	4,583	26	2,768	11	1,815	-	-	-	-

Notes: (1) A scientific evaluation grant for $40,000 is excluded.
(2) The three Minority Biomedical Support (S06) research grants are funded by an intraagency agreement. They are excluded in the number count but are included in the dollar figure.

NICHD-OPE-PAS
July 2, 1979

Research Accomplishments

Selected scientific accomplishments during FY 79 pertinent to the Branch's six problem oriented health areas are highlighted in the following paragraphs:

Dr. D.A. Fisher of Harbor General Hospital, California, is studying the maturration of the thyroid hormone system and the hormonal regulation of neonatal thermogenesis. His work has established the concept of fetal thyroid autonomy and has shown a two-phase increase in serum T3 at the time of parturition. The prenatal component appears to be cortisol dependent and the understanding of the early postnatal T3 surge may clarify the regulatory mechanisms for neonatal thermoregulation and adaptation.

Dr. A.S. Yeager at Stanford University, California, continues to document the effect that blood transfusion from cytomegalovirus (CMV) seronegative donors can markedly diminish the significant morbidity associated with CMV infections in newborn infants. The parallel assessment of humoral and cellular immunity in relation to the severity of CMV infections will permit new approaches for treatment of infected infants. In addition, the practicality for a blood bank to routinely screen blood units for CMV antibody is being explored.

Dr. M.J. Purves and staff at the University of Bristol, England, studying respiratory control mechanisms in fetal and newborn sheep, have identified that spontaneous activity in fetal cortex evoked potentials are affected by hypoxia. They have also demonstrated that carotid chemoreceptors, involved in respiratory control, are suppressed in fetal life and become activated at or shortly after birth.

Dr. J.D. Funkhouser and staff at the University of South Alabama, are investigating the effects of glucocorticoid hormones on fetal lung development, both in vitro and in vivo. Results obtained are providing clues to the identification of physiologic processes which affect lung development. These studies should broaden our understanding of the etiology of respiratory distress syndrome (RDS) and contribute to new therapeutic approaches.

Dr. R.A. Rhoades at Indiana University, Indiana, is investigating nutritional and hormonal interactions in lung development. He has examined the effect of maternal diabetes and nutrition on newborn lungs and pointed out that hormonal imbalance and/or altered substrate supply may be linked to onset of the respiratory distress syndrome.

Dr. J.W. Miller and associates at the University of Minnesota, Minnesota, are examining the possibility of using amino acid analogs as precursors to form new hormone peptides in situ which in turn could be employed to regulate the release of oxytocin in the neurohypophysis. Their studies on the oxytocin levels in fetal and newborn rat pups could provide evidence that fetal hormone release during parturition could contribute to the initiation of labor.

Dr. H.D. Niall and team of the University of Melbourne, Australia, are examining the chemical features of the relaxin molecule responsible for its biological properties. Because relaxin plays an important role in facilitating parturition, perhaps influencing the actual timing of the onset of labor, this study has significant implications for the treatment of both difficult labor and the premature initiation of labor.

Dr. T.H. Shepard of the University of Washington, Washington, continues to monitor and assess the incidence and type of defect in embryonic and fetal materials from spontaneous abortions. He has demonstrated that monitoring at the fetal level has two distinct advantages; (1) an increase in fetal malformations following a new environmental teratogen can precede, by at least six months, an identifiable epidemic increase in newborns, and (2) due to the shorter period between identification of a defect and the presumed time of insult, maternal recall is more accurate. It was observed that some pregnancies resulting in anencephaly were complicated by hyperthermia at the time of neurotube closure pointing to a possible etiologic factor.

Dr. S.A. Roth and staff at the Johns Hopkins University, Maryland are studying the phenomenon of morphogenesis in embryonic and pathologic systems. Studies have uncovered biochemical evidence for the localization on cell surfaces of endogenous glycosyltranserase activities, which may explain their potential role in cell recognition, migration, and preferential adherences. These findings may provide an important rationale toward understanding the occurence of birth defects.

Dr. L.A. Herzenberg and staff at Stanford University, California are studying the regulation of immunoglobulin isotype production by subclasses of T lymphocytes. They have succeeded in inducing allotype suppression using cell-free material and, therefore, a biochemical analysis of the basis for this inhibition is possible. With the development of clones of cells of the allotype-specific T suppressor (using hybrid cell-cell infusion techniques), specific and pure biological labels are produced in large quantities. This technique will permit inquiries into the mechanism of suppression in the young. It will in turn enhance our basic understanding of the processes of immunoglobulin synthesis and determine ways by which they may be altered by external manipulation.

At Vanderbilt University, Nashville, Tennessee, Dr. M.T. Stahlman and colleagues aim to define the chemoreflexes for apnea and swallowing and to determine whether these reflexes relate to recurrent apnea of prematurity, SIDS, and neonatal aspiration syndromes. Recent studies have demonstrated that, in newborn lambs, marked apnea can result from acid stimuli comparable to the acidic contents of the stomach. Repeated exposure of the laryngeal epithelial tissue to such acid solutions resulted in severe laryngeal epithelial cell damage similar to tissue injury observed in some cases of SIDS. Aspiration of feedings into the lungs was an apparent result of the observed tissue damage. These findings suggest that chronic exposure of laryngeal epithelium to acid solution may result in tissue damage that will permit aspiration of stomach contents to the lungs, and possibly sudden death.

At the University of Hawaii at Honolulu, Dr. Scott Halstead is studing infection enhancement in infants resulting from sequential infection by two types of dengue virus as a model for the etiology of sudden and unexpected death in infancy. Infants who are sequentially infected by the dengue viruses often die as a result of dengue shock syndrome (DSS). Infants who succumb to SIDS and to DSS show a similar age distribution pattern. It may be that pathogenetic mechanisms which trigger DSS and SIDS events are analagous. Dengue Shock Syndrome appears to occur because of an anaphylactic-like reaction resulting from the interaction between dengue infected mononuclear phagocytes and the organism's immune response(s). Dr. Halstead and his colleagues are attempting to identify components in the immune response to dengue infection in rhesus monkeys. A search will then be initiated for mediators of inflammation in cultures of immunologically attacked dengue infected monkey and human mononuclear phagocytes.

Dr. Arthur J. Miller, of the University of California Medical Center, San Francisco is using the rat as an experimental model to determine whether mild hypoxia, repeatedly experienced, can alter development of neuronal tissue in three brain stem regions: the nucleus tractus solitarius, which is the primary sensory nucleus of the upper respiratory tract; the nucleus coeruleus, which is involved in autonomic control; and the gigantocellular tegmental field of the pontine reticular formation, which is involved in rapid eye movement sleep. There exists a variety of evidence in support of the hypothesis that lagging neuronal development in the control of both sleep and respiration could be important in the etiology of SIDS. The continuing work of this investigator could contribute significantly to defining the relationship of synaptic growth of the neuron to effective control of sleep and of respiration and possibly to the occurrence of sudden death.

Major Research Programs

Major Research Programs (MRP) are program grants which promote and support multidisciplinary research efforts to develop new knowledge about diseases and disorders of pregnancy and infants. The Pregnancy and Infancy Branch is participating in the Institute's Major Research Program activities. It is responsible for the administration of six MRP's. Four of a total of seven MRP's are concerned with the maternal-infant health problem of diabetes mellitus in pregnancy. Other MRP's are involved with investigating the problems of (1) prematurity and the initiation of human parturition; (2) the sudden infant death syndrome; and (3) maternal smoking, fetal hypoxia, and neonatal outcome. The seven MRP's are located at the University of Texas; University of New Mexico; Northwestern University; Brown University; Case Western Reserve University; University of Maryland; and College of Physicians and Surgeons, Columbia University.

The first annual meeting of MRP directors took place in Bethesda, March 29-30, 1979. The individual programs presented a summary of their objectives and scope followed by a discussion of future plans. The objectives and accomplishments of each MRP are highlighted in the following paragraphs:

Dr. P.C. MacDonald and his colleagues at the University of Texas are pursuing studies on the prevention of prematurity by examining the mechanisms involved in the initiation of human labor. Much attention has been directed to the metabolism of the human fetal membranes with the view that these structures may play a metabolic as well as a mechanical role in the onset of parturition. These investigators found that fetal membranes are strikingly enriched in the obligate precursor of prostaglandins, arachidonic acid. Further studies determined that phosphatidylethanolamine is the principal precursor form from which arachidonic acid is liberated, and during labor there is an increased content of this specific fatty acid. The team has also identified a unique phospholipase A2 in the fetal membranes with substrate specificity for the identified compounds. Another area of intense research is to define the control ofthe unique endocrine system of the human fetus. In organ culture, it was determined that lipoprotein cholesterol is required for maximum stimulation of steroids. The preferred lipoprotein for the fetal adrenal is the low density lipoprotein (LDL) and efforts are presently concentrating on defining the factors which control their rate of synthesis.

Dr. R. Greenberg and his team at the University of New Mexico are coordinating efforts to study: (1) metabolic variants associated with diabetes mellitus based on observations of developmental events in animals, established disease patterns in humans, effect of maternal diabetes on developing organisms, and and mechanisms leading to the diabetic state in both animals and humans. An analysis of pregnancy study records in Pima Indians has shown that glucose tolerance in the third trimester is predictive of stillbirths and infant mortality and that in infants of diabetic mothers an inverse correlation exists between birth weight and occurrence of congenital defects. Other studies are measuring insulin receptors on red blood cells in rats where an increase in maximal binding of insulin was found after fasting the animals for 24 hours. In humans, it was determined that the affinity of these receptors for insulin is markedly diminished in infants of diabetic mothers. Preliminary studies seem to indicate that in some premature infants who were hyperglycemic, the increase in blood glucose appears related to stress phenomena associated with decreased glucose utilization. Progress has been achieved in defining the kinetics of peritoneal insulin delivery in somatostatin infused dogs which will facilitate the design of a long term implantable insulin delivery system for clinical indications.

Dr. N. Freinkel and collaborators at Northwestern University Medical School, are studying diabetes mellitus in pregnancy, specifically as it relates to maternal-child welfare, preventive medicine, therapeutics, and proper clinical management of the disease. One important aspect has been to determine possible diagnosis of macrosomia in utero as a first step to recognize and correct responsible maternal factors. For this purpose, ultrasound to measure fetal abdominal circumference and biparital diameter relationships in a serial fashion has proven successful to pinpoint intrauterine macrosomic propensities from week 28 onward. The research question being addressed is to determine whether normalization of maternal metabolism at this time can interrupt full expression of the process.

At Brown University, Dr. W. Oh and collaborators have studied in newborn lambs the effect of hyperviscosity on plasma glucose and endogenous production of glucose. It produced a significant reduction in glucose which did not appear to be accompanied by any alteration in the insulin secretion rate. In studies of bilirubin and fatty acids in infants of diabetic mothers, it has been found that these infants do not have free fatty acid concentration high enough to cause significant displacement of bilirubin from albumin. In a system with intact fat cells, these investigators also showed that bilirubin inhibits triglyceride lipase mediated lipolysis and increased glucose consumption by fat cells without increasing glucose transport. In addition, studies of placentas from diabetic mothers were found to have 50 per cent more infarcts and 47 per cent more secretory granules in the syncytial trophoblast.

Dr. P. Adam with a group of investigators at Case Western Reserve University, are completing 2 years of research in "disordered fetal metabolism, antenatal intervention." Urea production was determined and compared between non-pregnant and pregnant women. Pregnancy seems to attenuate urea production which sustains the hypothesis that aminoacids or nitrogen are preserved for synthetic processes in the mother and her fetus. Further studies will look into glucose turnover and formation of glucose from amino acids in pregnant diabetic women. Metabolic studies of low-birth weight infants have shown that 1) plasma glucose concentration correlates with glucose infusion rate; 2) plasma insulin correlates directly with plasma glucose concentration with no alteration induced by addition of amino acids; 3) the rate of early growth retardation continues up to 2 1/2 weeks after delivery despite careful increase in the infusion rate. The development of predictive endocrinologic methods for identification of fetal death risks prior to delivery is underway. A diminished maternal estradiol response to infusion of dehydroepiandrosterone seems to be indicative of abnormal births. Studies in the perceptual development of infants have shown high correlation between infant recognition scores at 16, 22 and 28 weeks of age and later vocabulary tests.

Dr. A. Steinschneider and his team at the University of Maryland Medical School are continuing their multidisciplinary, multifaceted examination of various facets of the sudden infant death syndrome. Newborn evaluations are continuing and risk profile scores are obtained on all infants. Due to the blind characteristics of the study design, no results will be available until certain set goals are reached. Data are collected on the effects of ambient temperature on neonatal studies performed within the first and fourth weeks of life. Plans are being developed to evaluate the impact on the nuclear family of a SIDS death.

Dr. L.S. James and team at Columbia University Presbyterian Medical Center, are initiating a research program to provide better methods for the detection and assessment of fetal hypoxia and to determine the ways in which maternal smoking adversely affects the fetus. The cardiovascular, respiratory, and biochemical responses of the fetus to both hypoxia and maternal smoking will be examined during development. The type of growth retardation caused by smoking, the timing of this effect, and the potential for the fetus to recover will be determined.

Sudden Infant Death Syndrome

During FY 1979, the Pregnancy and Infancy Branch continued to strengthen the Institute's sudden infant death syndrome (SIDS) program. This program is a broadly based research effort that aims to understand the underlying mechanisms of the syndrome, to determine its etiology, to identify infants at risk, to explore preventive approaches, to inform the scientific and lay communities about SIDS, and to stimulate scientists to direct their investigative efforts to the solution of this complex biomedical problem.

The Institute's seven SIDS research emphasis areas continue to be the focus of its investigative effort. These areas include: (1) developmental neurophysiology with emphasis on sleep patterns and associated autonomic disturbances which may interfere with vital functions; (2) respiratory, cardiac, and laryngeal responses to specific stimuli; (3) metabolic, thermal, and endocrine factors; (4) perinatal development of immune response mechanisms and infectious disease processes; (5) psychological stresses experienced by parents and siblings when a sudden, unexpected, and unexplained infant death occurs; (6) epidemiologic, environmental, and genetic aspects, and (7) anatomic pathology.

The 96th Congress is considering passage of a bill (S.497) that would continue the requirement that the Secretary, Department of Health, Education and Welfare, provide adequate funding for NICHD research activities in SIDS. In fiscal year 1978, the latest year for which an annual total is available, the Institute obligated $8.7 million to support 97 research projects. Of this total, $4.0 million supported 34 research projects specifically concerned with SIDS in accordance with the provisions of Section 2(c) of P.L. 93-270. The remaining $4.7 million provided support for 63 projects generally related to the problem of SIDS.

As a result of Institute supported investigations of SIDS between 1972 and 1978, there is increasing support for the idea that the victims of crib death are not completely healthy and their deaths not as inexplicable as once thought. Many of these infants have subtle physiologic defects of a neurologic, cardiorespiratory, and/or metabolic nature which may reflect impediments in the victim's maturational processes. Moreover, there is increasing evidence that the pathogenesis of the syndrome is not through a single mechanism as previously believed. Indications that these infants have pre-existing difficulties include anatomic pathologic findings suggestive of chronic stress and hypoxia; abnormalities in sleep state, cardiorespiratory function, and tissue oxygen utilization; postnatal growth retardation; and the infant's temperament and behavioral patterns between birth and death. Currently suggested etiologic mechanisms revolve around interrelated infectious disease processes and developmental, pathophysiologic, metabolic, and environmental phenomena. A number of these phenomena are closely related to chronic stress and hypoxic states.

In FY 1978, the Institute initiated a Cooperative Epidemiologic Study of SIDS Risk Factors. The Study involves six data collection centers located in New York City, upstate New York, Chicago, St. Louis, northern California, and

Seattle; a data coordinating center at the University of Washington in Seattle; and a pathology processing laboratory in the Medical Examiner's Office in San Francisco. The purpose of this study is to focus on the development of a risk prediction algorithm which can be applied in the first week of life. Case-control comparisons for each factor studied will determine the extent of SIDS risk associated with the factor. At least 759 cases of SIDS, defined by a common necropsy protocol, will be possible to identify high risk infants on the basis of information available at birth and in the period shortly after birth. Entry of cases into the study began in early October 1978 and will end on the last day of December, 1979. Development of strategies for data analysis began in July 1979. It is expected that a final report in the form of a monograph will be ready for publication by early 1982. Selected highlights of investigator-initiated research on SIDS carried out during FY 1979 were highlighted previously in this report.

Smoking in Pregnancy

In FY 1979 the Branch participated in the Department's initiative on the Health Consequences of Smoking. Staff assumed responsibility for the organization and preparation of a manuscript updating current knowledge on the effects of maternal smoking on pregnancy and infancy. This document has been published as part of the 1979 Surgeon General's report on the Health Consequences of Smoking. The Branch also sponsored a research planning worskshop on maternal smoking, pregnancy outcome, and infant health. In order to stimulate investigator initiated research in this area, staff has issued an RFA entitled "Maternal Smoking and Pregnancy Outcome".

Conferences, Staff Activities, and Publications

During FY 79, the Branch provided partial support for the Perinatal Research Society's Interdisciplinary Conference on Perinatology. It also sponsored a research planning workshop entitled: The Health Consequences of Smoking During Pregnancy: Critical Questions for Research in December, 1978.

Staff members served as Institute representatives at a number of professional organizations' meetings including the Society for Gynecologic Investigation, Society for Pediatric Research, and the American Pediatric Society as well as the American Academy of Pediatrics Committee of the Fetus and Newborn and Committee on Drugs.

Branch publications include:

Hasselmeyer, E.G., Meyers, M.B., Catz, C., and Longo, L.D.: Pregnancy and Infant Health. In Smoking and Health. A Report of the Surgeon General. U.S. Department of Health, Education, and Welfare, Public Health Service Publication No. 79-50066. Washington, D.C., U.S. Government Printing Office, 1979, Chapter 8, pp. 1-93.

Giacoia, G.P., and Catz, C.S.: Drugs and Pollutants in Breast Milk. Clinics in Perinatology. 6: 181-196, 1979.

Catz, C., and Yaffe, S.J., Developmental Pharmacology. In Falkner, F., and Tanner, J.M., (Eds.): Human Growth Treatise. New York, Plenum Publishing Corp., 1978, Vol. I, pp. 125-144.

Personnel

At the beginning of FY 79, the Branch was assigned five professional positions, one secretary, one clerk stenographer, one clerk-dictating machine transcriber, and two part-time clerk typists. Two of the professional positions were staffed with persons holding Ph.D. degrees; and the other one by a medical officer qualified in pediatrics. Four vacancies, two in the professional staff positions and two in the secretarial staff, ccurred during the year. With the ending of FY 79, a reorganization within the CRMC has combined the Pregnancy and Infancy Branch with the Developmental Biology and Nutrition Branch into a newly established Branch entitled Clinical Nutrition and Early Development Branch.

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Pregnancy and Infancy Branch
Contract and Collaborative Research

Contract Number: NO1 HD 1-2088

Contract Title: Non-Human Primate Colony

Contractor: University of California, Davis, California

Money Allocated: $209,018 (October 1, 1978 to September 30, 1979)

Objectives: Under this contract, a colony of Rhesus monkeys (Macaca mulatta) of known medical, reproductive and genealogical history is being kept and developed at the California Primate Research Center, University of California at Davis. This colony serves as a resource of research animals of known quality from a standardized environment for investigators whose research is funded by the NICHD.

Procedures: Animals of different ages including dated pregnancies, embryonic and fetal material, neonates of known gestation, mother-infant pairs, juveniles of known age, as well as certain biopsy material, tissues, and fluids are being made available to selected investigators, all of whom must be directly supported by NICHD. Application for animals is competitive and allocation is determined by an advisory committee which also advises on colony and contract management. Animals are shipped to recipient scientists throughout the country; a limited number of investigators can also be accommodated to visit facilities on-site. Biological and behavioral data profiles are compiled and incorporated into the computerized record of each animal. This information is available to recipient investigators.

Significance: Demand for animals from this resource reflects the use of Rhesus monkeys as a model for the human in biomedical research.

Proposed Course: The size of the colony, stabilized at 335 animals, is adequate for the projected demand of 80-100 pregnancies per year plus the need of replacement breeder stock. During the present year an RFP was issued to assess sources available for the management of this colony. The responses received were evaluated for their technical and business content. A final review and decision as to the selection of contractor has not yet been reached.

Project Officer: Charlotte Catz, M.D.

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Contract and Collaborative Resarch

Contract Number: NO1 HD 2-2777

Contract Title: Development of Sleep and Cardiopulmonary Regulations Within Sleep: Clinical Studies of a Functional Mechanism for Risk of the Sudden Infant Death Syndrome (SIDS)

Contractor: Professional Staff Association of LAC/USC Medical Center

Money Allocated: $38,776 (November 1, 1978 to March 31, 1979)

Objectives: To describe the early maturation of sleep "states" and cardiopulmonary function during the various "states" in groups of newborns at high and low risk for SIDS. This contract is oriented exclusively toward data collection. Data analysis is a major component of its companion contract at UCLA (NO1 HD 2-2810).

Findings: Large amounts of physiological data were collected to evaluate the early maturation of sleep states and cardiopulmonary function during state. The three study groups consisted of (1) twenty-five subsequent siblings of SIDS cases; (2) twenty-five healthy normal newborns; and (3) ten "near miss" infants. The first two groups were recruited into the study prenatally and monitored at 32 and 36 weeks gestation during the first week after birth, and at monthly intervals during the first six months of life. Each all-night monitoring session lasted 12 hours during which 12 physiological variables were recorded continuously. Data collection ended September 1977.

Significance: The cause of SIDS is at present unknown. A major etiologic hypothesis suggests a delayed maturation of physiologic responsiveness in the ontogeny of sleep states. This project is developing the data base upon which this hypothesis can be tested by measuring and recording physiological variables at different stages of sleep during the first six months of life when more than 80 percent of SIDS cases occur. This study is an integral part of the Institute's SIDS research contract program to develop further knowledge on the causes and underlying mechanisms of SIDS.

Proposed Course: A monograph is being prepared which will describe details of the project and document results. This document will be of value in future SIDS risk prediction studies. Publication of the monograph is planned for 1980 - 1981.

Project Officer: Eileen G. Hasselmeyer, Ph.D.

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Contract and Collaborative Research

Contract Number: NO1 HD -7-2839

Contract Title: Data Coordinating Center for the NICHD Cooperative Epidemiologic SIDS Risk Factors Study

Contractor: University of Washington
Seattle, WA

Money Allocated: $130,910 (January 1, 1979 to December 31, 1979)

Objectives: This contract serves as the Data Coordinating Center to the NICHD Cooperative Epidemiologic SIDS Risk Factors Study. It is responsible for the processing, management, and analyses of data collected by the participating Study Centers.

Findings: The study design is completed. Protocols, questionnaires, medical record abstraction forms and reporting forms for the Study Centers have been developed and are in use.

Significance: The sudden infant death syndrome is the leading cause of death in the United States between one month and one year of life. This study is an integral part of the NICHD SIDS research contract program to understand the causes and underlying mechanisms of SIDS and to identify infants at risk.

Proposed Course: Data collection began on October 9, 1978 and will be completed on December 31, 1979. Data analysis will begin in June, 1980 and will proceed for an additional 12 months.

Project Officer: Eileen G. Hasselmeyer, Ph.D.

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Contract and Collaborative Research

Contract Number: N01 HD 7-2839

Contract Title: Data Collection Center for the NICHD Cooperative Epidemiologic SIDS Risk Factors Study

Contractor: University of Seattle
Seattle, Washington

Money Allocated: $16,000 (January 1, 1979 - December 31, 1979)

Objectives: This contract is an essential component of the NICHD Cooperative Epidemiologic SIDS Risk Factors Study involving 6 data collecting centers and 1 data coordinating center. The purpose of this cooperative study is to focus on the development of a risk prediction algorithm which can be applied in the first week of life. The study design is case-control to be maximally efficient in identification of risk factors; it is also population based (complete case ascertainment within defined study populations) to enable quantification of the risk associated with any identified risk factor. An estimated 750 - 1,000 possible SIDS cases will enter the study and 2 matched living controls will be selected for each possible SIDS case.

The six data collecting centers include N01 HD 7-2839, University of Washington, Seattle; N01 HD 7-2840, University of California, Davis; N01 HD 7-2841, Health Research Inc., Albany, New York; N01 HD 7-2842, Medical and Health Research Association of New York City; N01 HD 7-2843 Loyola University of Chicago; N01 HD 7-2844, St Louis Regional Maternal and Child Health Inc. The data coordinating center is located at the University of Washington (N01 HD 7-2839).

Findings: Data collection for the study began on October 9, 1978. No serious problems have been encountered during acquisition of data through the use of parent interviews and by collection of tissues from SIDS victims at autopsy. An improved form for the abstraction of medical records has been completed and distributed to the six Data Collection Centers.

Significance: The sudden infant death syndrome is the leading cause of death in the United States between one month and one year of life. This study is an integral part of the NICHD SIDS research contract program to understand the causes and underlying mechanisms of SIDS and to identify infants at risk.

Proposed Course: Entry of SIDS cases into the study will end December 31, 1979. Cleaning and editing of data by the Data Coordinating Center should be completed by June 1980. Development of strategies for data analysis will begin in August 1979. A final report on approaches to data analyses will be completed by March 1980. Study results will be reported in a monograph to be available in 1981.

Project Officer: Eileen G. Hasselmeyer, Ph.D.

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Contract and Collaborative Research

Contract Number: N01 HD 7-2840

Contract Title: Data Collection Center for the NICHD Cooperative Epidemiologic SIDS Risk Factors Study

Contractor: University of California
Davis, California

Money Allocated: $91,785 (January 1, 1979 - December 31, 1979)

Objectives: This contract is an essential component of the NICHD Cooperative Epidemiologic SIDS Risk Factors Study involving 6 data collecting centers and 1 data coordinating center. The purpose of this cooperative study is to focus on the development of a risk prediction algorithm which can be applied in the first week of life. The study design is case-control to be maximally efficient in identification of risk factors; it is also population based (complete case ascertainment within defined study populations) to enable quantification of the risk associated with any identified risk factor. An estimated 750 - 1,000 possible SIDS cases will enter the study and 2 matched living controls will be selected for each possible SIDS case.

The six data collecting centers include N01 HD 7-2839, University of Washington, Seattle; N01 HD 7-2840, University of California, Davis; N01 HD 7-2841, Health Research Inc., Albany, New York; N01 HD 7-2842, Medical and Health Research Association of New York City; N01 HD 7-2843 Loyola University of Chicago; N01 HD 7-2844, St Louis Regional Maternal and Child Health Inc. The data coordinating center is located at the University of Washington (N01 HD 7-2839).

Findings: Data collection for the study began on October 9, 1978. No serious problems have been encountered during acquisition of data through the use of parent interviews and by collection of tissues from SIDS victims at autopsy. An improved form for the abstraction of medical records has been completed and distributed to the six Data Collection Centers.

Significance: The sudden infant death syndrome is the leading cause of death in the United States between one month and one year of life. This study is an integral part of the NICHD SIDS research contract program to understand the causes and underlying mechanisms of SIDS and to identify infants at risk.

Proposed Course: Entry of SIDS cases into the study will end December 31, 1979. Cleaning and editing of data by the Data Coordinating Center should be completed by June 1980. Development of strategies for data analysis will begin in August 1979. A final report on approaches to data analyses will be completed by March 1980. Study results will be reported in a monograph to be available in 1981.

Project Officer: Eileen G. Hasselmeyer, Ph.D.

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Contract and Collaborative Research

Contract Number: NO1 HD 7-2841

Contract Title: Data Collection Center for the NICHD Cooperative Epidemiologic SIDS Risk Factors Study

Contractor: Health Research Inc.
Albany, New York

Money Allocated: $89,150 (January 1, 1979 - December 31, 1979)

Objectives: This contract is an essential component of the NICHD Cooperative Epidemiologic SIDS Risk Factors Study involving 6 data collecting centers and 1 data coordinating center. The purpose of this cooperative study is to focus on the development of a risk prediction algorithm which can be applied in the first week of life. The study design is case-control to be maximally efficient in identification of risk factors; it is also population based (complete case ascertainment within defined study populations) to enable quantification of the risk associated with any identified risk factor. An estimated 750 - 1,000 possible SIDS cases will enter the study and 2 matched living controls will be selected for each possible SIDS case.

The six data collecting centers include NO1 HD 7-2839, University of Washington, Seattle; NO1 HD 7-2840, University of California, Davis; NO1 HD 7-2841, Health Research Inc., Albany, New York; NO1 HD 7-2842, Medical and Health Research Association of New York City; NO1 HD 7-2843 Loyola University of Chicago; NO1 HD 7-2844, St Louis Regional Maternal and Child Health Inc. The data coordinating center is located at the University of Washington (NO1 HD 7-2839).

Findings: Data collection for the study began on October 9, 1978. No serious problems have been encountered during acquisition of data through the use of parent interviews and by collection of tissues from SIDS victims at autopsy. An improved form for the abstraction of medical records has been completed and distributed to the six Data Collection Centers.

Significance: The sudden infant death syndrome is the leading cause of death in the United States between one month and one year of life. This study is an integral part of the NICHD SIDS research contract program to understand the causes and underlying mechanisms of SIDS and to identify infants at risk.

Proposed Course: Entry of SIDS cases into the study will end December 31, 1979. Cleaning and editing of data by the Data Coordinating Center should be completed by June 1980. Development of strategies for data analysis will begin in August 1979. A final report on approaches to data analyses will be completed by March 1980. Study results will be reported in a monograph to be available in 1981.

Project Officer: Eileen G. Hasselmeyer, Ph.D.

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Contract Number: NO1 HD 7-2842

Contract Title: Data Collection Center for the NICHD Cooperative Epidemiologic SIDS Risk Factors Study

Contractor: Medical and Health Research Association of New York City
New York, New York

Money Allocated: $125,135 (January 1, 1979 - December 31, 1979)

Objectives: This contract is an essential component of the NICHD Cooperative Epidemiologic SIDS Risk Factors Study involving 6 data collecting centers and 1 data coordinating center. The purpose of this cooperative study is to focus on the development of a risk prediction algorithm which can be applied in the first week of life. The study design is case-control to be maximally efficient in identification of risk factors; it is also population based (complete case ascertainment within defined study populations) to enable quantification of the risk associated with any identified risk factor. An estimated 750 - 1,000 possible SIDS cases will enter the study and 2 matched living controls will be selected for each possible SIDS case.

The six data collecting centers include NO1 HD 7-2839, University of Washington, Seattle; NO1 HD 7-2840, University of California, Davis; NO1 HD 7-2841, Health Research Inc., Albany, New York; NO1 HD 7-2842, Medical and Health Research Association of New York City; NO1 HD 7-2843 Loyola University of Chicago; NO1 HD 7-2844, St Louis Regional Maternal and Child Health Inc. The data coordinating center is located at the University of Washington (NO1 HD 7-2839).

Findings: Data collection for the study began on October 9, 1978. No serious problems have been encountered during acquisition of data through the use of parent interviews and by collection of tissues from SIDS victims at autopsy. An improved form for the abstraction of medical records has been completed and distributed to the six Data Collection Centers.

Significance: The sudden infant death syndrome is the leading cause of death in the United States between one month and one year of life. This study is an integral part of the NICHD SIDS research contract program to understand the causes and underlying mechanisms of SIDS and to identify infants at risk.

Proposed Course: Entry of SIDS cases into the study will end December 31, 1979. Cleaning and editing of data by the Data Coordinating Center should be completed by June 1980. Development of strategies for data analysis will begin in August 1979. A final report on approaches to data analyses will be completed by March 1980. Study results will be reported in a monograph to be available in 1981.

Project Officer: Eileen G. Hasselmeyer, Ph.D.

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Contract Number: NO1 HD 7-2843

Contract Title: Data Collection Center for the NICHD Cooperative Epidemiologic SIDS Risk Factors Study

Contractor: Loyola University of Chicago
Chicago, Illinois

Money Allocated: $104,028 (January 1, 1979 - December 31, 1979)

Objectives: This contract is an essential component of the NICHD Cooperative Epidemiologic SIDS Risk Factors Study involving 6 data collecting centers and 1 data coordinating center. The purpose of this cooperative study is to focus on the development of a risk prediction algorithm which can be applied in the first week of life. The study design is case-control to be maximally efficient in identification of risk factors; it is also population based (complete case ascertainment within defined study populations) to enable quantification of the risk associated with any identified risk factor. An estimated 750 - 1,000 possible SIDS cases will enter the study and 2 matched living controls will be selected for each possible SIDS case.

The six data collecting centers include NO1 HD 7-2839, University of Washington, Seattle; NO1 HD 7-2840, University of California, Davis; NO1 HD 7-2841, Health Research Inc., Albany, New York; NO1 HD 7-2842, Medical and Health Research Association of New York City; NO1 HD 7-2843 Loyola University of Chicago; NO1 HD 7-2844, St Louis Regional Maternal and Child Health Inc. The data coordinating center is located at the University of Washington (NO1 HD 7-2839).

Findings: Data collection for the study began on October 9, 1978. No serious problems have been encountered during acquisition of data through the use of parent interviews and by collection of tissues from SIDS victims at autopsy. An improved form for the abstraction of medical records has been completed and distributed to the six Data Collection Centers.

Significance: The sudden infant death syndrome is the leading cause of death in the United States between one month and one year of life. This study is an integral part of the NICHD SIDS research contract program to understand the causes and underlying mechanisms of SIDS and to identify infants at risk.

Proposed Course: Entry of SIDS cases into the study will end December 31, 1979. Cleaning and editing of data by the Data Coordinating Center should be completed by June 1980. Development of strategies for data analysis will begin in August 1979. A final report on approaches to data analyses will be completed by March 1980. Study results will be reported in a monograph to be available in 1981.

Project Officer: Eileen G. Hasselmeyer, Ph.D.

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Contract Number: NO1 HD 7-2844

Contract Title: Data Collection Center for the NICHD Cooperative Epidemiologic SIDS Risk Factors Study

Contractor: St. Louis Regional Maternal and Child Health Inc.
St. Louis, Missouri

Money Allocated: $64,537 (January 1, 1979 - December 31, 1979)

Objectives: This contract is an essential component of the NICHD Cooperative Epidemiologic SIDS Risk Factors Study involving 6 data collecting centers and 1 data coordinating center. The purpose of this cooperative study is to focus on the development of a risk prediction algorithm which can be applied in the first week of life. The study design is case-control to be maximally efficient in identification of risk factors; it is also population based (complete case ascertainment within defined study populations) to enable quantification of the risk associated with any identified risk factor. An estimated 750 - 1,000 possible SIDS cases will enter the study and 2 matched living controls will be selected for each possible SIDS case.

The six data collecting centers include NO1 HD 7-2839, University of Washington, Seattle; NO1 HD 7-2840, University of California, Davis; NO1 HD 7-2841, Health Research Inc., Albany, New York; NO1 HD 7-2842, Medical and Health Research Association of New York City; NO1 HD 7-2843 Loyola University of Chicago; NO1 HD 7-2844, St Louis Regional Maternal and Child Health Inc. The data coordinating center is located at the University of Washington (NO1 HD 7-2839).

Findings: Data collection for the study began on October 9, 1978. No serious problems have been encountered during acquisition of data through the use of parent interviews and by collection of tissues from SIDS victims at autopsy. An improved form for the abstraction of medical records has been completed and distributed to the six Data Collection Centers.

Significance: The sudden infant death syndrome is the leading cause of death in the United States between one month and one year of life. This study is an integral part of the NICHD SIDS research contract program to understand the causes and underlying mechanisms of SIDS and to identify infants at risk.

Proposed Course: Entry of SIDS cases into the study will end December 31, 1979. Cleaning and editing of data by the Data Coordinating Center should be completed by June 1980. Development of strategies for data analysis will begin in August 1979. A final report on approaches to data analyses will be completed by March 1980. Study results will be reported in a monograph to be available in 1981.

Project Officer: Eileen G. Hasselmeyer, Ph.D.

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Contract and Collaborative Research

Contract Number: N01 HD 8-2845

Contract Title: Pathological Coordinating Laboratory (PCL) for the NICHD Cooperative Epidemiologic Study of SIDS Risk Factors

Contractor: Office of Medical Examiner
City and County of San Francisco
San Francisco, California

Money Allocated: $199,341 (September 30, 1978 through September 29, 1979)

Objectives: This contract serves as the Pathology Coordinating Laboratory to the NICHD Cooperative Epidemiologic SIDS Risk Factors Study. The Labortory provides essential services to ensure the accurate diagnosis of SIDS. The necropsy, a common gross dissection protocol, will be followed to dichotomize the dead infants into "definitely not SIDS" and "eligible for further study". The contractor will process tissues submitted by six Study Centers and will produce a set of stained slides for each infant entered into the study.

Findings: A common gross dissection protocol has been developed. Medical Examiners serving the six Study Centers have been following the protocol since the start of data collection (October 9, 1978). As of June 30, 1979 tissues from 433 dead infants have been submitted to the PCL for processing. It is anticipated that cases from more than 900 infants who died suddenly and unexpectedly will be processed by June 30, 1980.

Significance: The sudden infant death syndrome is the leading cause of death in the United States between one month and one year of life. This study is an integral part of the NICHD SIDS research contract program to understand the causes and underlying mechanisms of SIDS and to identify infants at risk.

Project Officer: Eileen G. Hasselmeyer, Ph.D.

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Contract and Collaborative Research

Contract Number: NO1 HD 9-2808

Contract Title: Sleep Apnea and the Sudden Infant Death Syndrome: Developmental Correlates

Contractor: Syracuse University
Syracuse, New York

Money Allocated: $89,844 (November 1, 1978 to October 31, 1979)

Objectives: The objective of this project is to carry out a longitudinal follow-up of infants with established sleep apnea and their controls, including evaluation for respiratory instability within the first week of life, correlations of the amount of sleep apnea in the first and fourth weeks of life with measures of neurological and behavioral function, cry pattern spectographs, and mental and psychomotor developmental assessment.

Findings: Data collection ended December, 1977. Data are available for analysis regarding sleep apnea on 196 full-term infants, 83 subsequent siblings of SIDS victims, and 178 premature infants at 1 week and 4 weeks of age, as well as nine month Bayley developmental scores. Preliminary data analyses indicate that infants who had a high frequency of breathing pauses (apnea) of two seconds or greater duration had lower mental and motor scores on the Bayley scales than infants without evidence of apnea. Developmental differences appear to be definite at 9 months of age, but are equivocal at 2 years of age. Correlation analyses of data on sleep apnea of two seconds or greater duration and developmental test scores, and regression analyses of data on obstetrical and neonatal variables and developmental outcomes will contribute significantly to knowledge about developmental events antecedent to SIDS.

Significance: This study is an integral part of the Institute's SIDS research contract program to identify the causes and underlying mechanisms of SIDS. This proposal is developing new information that will help to identify infants at risk for SIDS and to extend our understanding of the impact of sleep apnea on development.

Proposed Course: Data analyses will continue with completion of the contract by December 1979.

Project Officer: Eileen G. Hasselmeyer, Ph.D.

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Contract and Collaborative Branch

Contract Number: NO1 HD 4-2810

Contract Title: Analysis of State Integration and Relation to Cardiopulmonary Functions in Infants at High and Low Risk for the Sudden Infant Death Syndrome (SIDS)

Contractor: University of California at Los Angeles

Money Allocated: $56,223 (November 1, 1978 to May 31, 1979)

Objectives: To describe the early maturation of sleep states and cardiopulmonary function in groups of newborn infants at high and low risk for SIDS, and to develop an animal model to test physiologic hypotheses of SIDS etiology. This contract emphasizes data analysis while its companion contract at USC (NO1 HD 4-2777) is for data collection.

Findings: Results to date support the concept that a faulty progression in the maturation of sleep and heart-lung control may contribute to the mechanisms of SIDS. The characteristics of the development of sleep have been described with greater physiologic variability observed in low risk infants. It was found that different developmental patterns characterize sleep and related cardiopulmonary functions in high and low risk infants; specific brain wave differences exist at particular ages during the first 6 months of life; sleep state cycling over night is different in high and low risk infants; sleep state can alter variations in heart and respiratory rates; a fixed sequence of sleep state occurs after feeding; a distinct developmental sequence of apnea incidence has been elucidated; high risk infants have a higher respiratory rate than low risk infants and a reduced incidence of apnea.

Data from kittens under different stress stages (oxygen and sleep deprivation), have revealed a "failure syndrome" associated with rearing in a low-oxygen atmosphere. In this syndrome, kittens with a high respiratory rate and few apnea spells become progressively unable to enter active sleep and eventually heart and lungs cease to function. These findings support the possible role of sleep-heart-lung-regulation in the etiology of SIDS.

Significance: The results of this contract will contribute to the development of highly refined estimates of SIDS risk.

Proposed Course: A monograph is being prepared which will describe details of the project and document results. This document will be of value in future SIDS risk prediction studies. Publication of the monograph is planned for 1980 - 1981.

Project Officer: Eileen G. Hasselmeyer, Ph.D.

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Contract and Collaborative Research

Contract Number: N01 HD 5-2855

Contract Title: Evaluation and Follow-up of Selected Respiratory, Cardiac and Neurophysiologic Parameters in Infants as Indicators of Risk for the Sudden Infant Death Sydnrome (SIDS)

Contractor: Montefiore Hospital and Medical Center, Bronx, New York

Money Allocated: Not to exceed $150,000 (September 30, 1979 - March 29, 1980)

Objectives: The objectives of this project are to study cardiorespiratory-neurophysiologic phenomena which may be relevant to the understanding of sudden infant death syndrome (SIDS) and to identifying infants at risk.

Findings: During the current year a great emphasis was placed on data reduction. Hand scoring of sleep and apnea has been completed for a total of 114 sessions which comprises 85 recordings of "near-miss" infants, 21 recordings of control babies and 8 recordings from the subsequent siblings of SIDS victims. Scorings entered into the computer are merged to generate a sleep-apnea report for day and night recordings. It was found that the number of apneas per hour occur more frequently at night and decrease with increasing age. The latter is more noticeable in daytime recordings. Work in time series analysis has begun and efforts were concentrated in developing relevant software for use in the automatic detection of apneas and in the study of respiratory and heart rate characteristics. A portable kit for home recordings has been readied for a final in-hospital test prior to utilization in the home with selected patients.

Significance: The sudden infant death syndrome is the leading cause of death in the United States between the ages of one month and one year. This proposal is developing new information that will help to identify infants at risk for SIDS. This study is an integral part of the Institute's SIDS research contract program to develop further knowledge on the causes and underlying mechanisms of SIDS.

Proposed Course: A renewal proposal is under consideration for FY 1980.

Project Officer: Charlotte Catz, M.D.

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Contract and Collaborative Research

Contract Number: N01 HD 5-2856

Contract Title: Evaluation and Follow-up of Selected Respiratory, Cardiac and Neurophysiologic Parameters in Infants as Indicators of Risk for the Sudden Infant Death Syndrome (SIDS)

Contractor: Columbia-Presbyterian Medical Center, New York, New York

Money Allocated: $415,866 (July 1, 1979 - June 30, 1980)

Objectives: The objectives of this project are to study cardiorespiratory-neurophysiologic phenomena which may be relevant to understanding the sudden infant death syndrome (SIDS) and identifying infants at risk.

Findings: Healthy infants and infants with "near-miss" episodes are monitored during sleep in a non-invasive manner. Data obtained include recordings of EEG, EOG, EMG, EKG, respiratory rate and respiratory volume. New statistical methods have been developed to determine the interrelationships between cardiac and respiratory parameters. The cardiac data show: 1) QT intervals normalized for heart rate differences in all infants is shorter during REM sleep, 2) QT was shorter at all ages in REM and quiet sleep states in the "near-miss" infant (reflects an increase in sympathetic tone or circulatory catecholamines), 3) RR intervals were always shorter in "near-miss" infants with a decrease in beat to beat variability. Respiratory data indicate maturational changes in ventilation and ventilatory pattern which appear to be under central control mechanisms. Findings indicate that in ventilation the inspiratory time (Ti) and expiratory time (Te) of a single breath may be controlled by different mechanisms. Maturation seems to have a stabilizing effect on breath to breath control of ventilation only in REM sleep. Breathing during sleep was found to be cyclic and possibly related to neural mechanism. A pilot study in puppies has demonstrated the feasibility of an animal model to determine ventilatory, cardiac, and sleep parameters and their relationships to blood gases and circulating chemical mediators.

Significance: The sudden infant death syndrome is the leading cause of death in the United States between the age of one month to one year. This proposal is developing new information that will help to identify infants at risk for SIDS. This study is an integral part of the Institute's SIDS research contract program to develop further knowledge on the causes and underlying mechanisms of SIDS.

Proposed Course: A renewal proposal is approved for FY 1980.

Project Office: Charlotte Catz, M.D.

NICHD Annual Report
October 1, 1978 through September 30, 1979
Developmental Biology and Nutrition Branch
Center for Research for Mothers and Children

This is the third and final Annual Report of the Developmental Biology and Nutrition Branch. The Branch was created on August 11, 1976, and was abolished officially as of June 1, 1979, when it joined the former Pregnancy and Infancy Branch to form the Clinical Nutrition and Early Development Branch, the largest branch of the Institute. Gilman D. Grave, M.D., continued to serve as Acting Chief of the DBN Branch during FY 79. Delbert H. Dayton, M.D., Thorsten A. Fjellstedt, Ph.D. and Anne K. Krey, M.S., who joined the Branch on November 27, 1978, are the other members who make up the professional staff. The support staff consists of Mrs. Kathryn I. Brown, Mrs. Kathleen T. Wieder and Ms. Lesia A. Myers.

The mission of the DBN Branch is to understand the mechanisms that operate during development to promote health and to prevent disease. Research supported by the Branch stresses the study of genetic, endocrine, metabolic, immunologic and nutritional antecedents of infant, childhood and adult disorders.

The staff of the DBN Branch emphasizes research in ten areas which encompass studies ranging from basic and molecular biology to clinical studies. The research programs and their share of the FY 79 budget for the DBN Branch are presented below:

1.	Cell Differentiation	23%
2.	Growth and Growth Factors	21%
3.	Developmental Human Genetics	12%
4.	Developmental Nutrition	10%
5.	Obesity and Childhood Antecedents of Adult Disease	10%
6.	Developmental Immunology	9%
7.	Infant Nutrition	7%
8.	Cultural and Behavioral Aspects of Nutrition	5%
9.	Developmental Gastroenterology	2%
10.	Malnutrition and the Immune Response	1%

The estimated budget for the DBN Branch in FY 79 amounts to $22.7 million. This represents an increase of 25% over last year's budget. From its inception the programs of the Developmental Biology and Nutrition Branch grew rapidly in size and number. During the life of the DBN Branch the staff initiated new research programs in the following areas: Infant Nutrition; Developmental Gastroenterology; Childhood Obesity and Nutritional Antecedents of Adult Disease; and Cultural and Behavioral Aspects of Nutrition.

As the programs of the Branch grew, their relative sizes remained constant, except for the nutrition programs. The total budget of these programs increased within three fiscal years from $4.7 million to $8.8 million. During this time, support of research in childhood obesity and nutritional antecedents of adult disease nearly doubled, and the programs in infant nutrition and developmental

gastroenterology trebled. These changes reflect a successful effort by the staff of the Branch to stimulate these important fields of research. In FY 79 a new program on Cultural and Behavioral Aspects of Nutrition was transferred to the Branch from the Office of Planning and Evaluation. This program represents a significant effort to stimulate an area of research that has traditionally received little attention from the NIH.

Each of the ten programs, their research priorities and some of their recent accomplishments are discussed below.

The Research Programs

1. Cellular Differentiation - The largest program within the DBN Branch is comprised of studies of cellular differentiation. Critically important to development is the process of cell differentiation. This development process has been studied by several investigators supported by the DBN Branch who employ insect models to elucidate organization and control of expression of genetic information, transport and storage of transcribed messages, and subsequent translation of the messages into tissue- and stage-specific proteins. Other Branch-supported investigators use avian and mammalian models for studies of the differential developmental expression of genetic information.

This year the DBN Branch awarded a Program Project grant to a group of investigators from the University of Michigan devoted to isolating and purifying the human genes that code for transfer RNA and ribosomal RNA by the use of recombinant techniques. These basic studies will aid in learning more about the variability of gene sequence within individuals, about gene organization, and about interactions between chromosomes.

Transcription of genes during development into specific RNA messages is currently being studied by a group of workers at Harvard who have used the recombinant DNA technique during the past year to improve understanding of normal and abnormal developmental expression of the collagen genes. Other investigators have quantified the synthesis, nuclear-cytoplasmic transport, and the use of various RNA messages, determined their sequence complexity, and have related the biosynthesis of mRNAs for specific proteins to particular developmental events. Expression of genes is now being studied during very early developmental stages. Currently under investigation are maternally-synthesized RNA messages which support protein synthesis right after fertilization. The long range objective of these investigations is understanding of how maternal factors influence the early expression of genes to yield cell specializations which determine the pattern of tissue and organ formation in later embryonic life.

2. Growth and Growth Factors - This program is concerned with basic and clinical aspects of human growth and development. Although some projects receive support that are devoted to somatic growth, skeletal age and gait development, the majority of the research funded by this program falls within the areas of developmental endocrinology and neuroendocrinology. Of particular interest are problems of growth hormone deficiency, pubertal onset, and the mechanisms of action of hormones that play directive or permissive roles during human

growth and development.

The DBN Branch currently supports ten projects on various aspects of growth hormone. Because of the scarcity of human growth hormone, the DBN Branch funds a project designed to isolate biologically active fragments of the growth hormone molecule. Eventually such peptide moieties may be synthesized, or cleaved from molecules of animal growth hormone, or even harvested from bacterial clones. Other projects on growth hormone are concerned with its production rates and patterns of release in normal and growth-retarded children.

The DBN Branch began support of several new studies in FY 79 on the control of pubertal onset. Certain parts of the brain have only recently been characterized as endocrinologic in nature, particularly the hypothalamus and the pineal organ. Other parts of the brain seem to act as a biological clock which signals the hypothalamus when to alter its feedback sensitivity to steroid hormones. Recent advances have shown that the pituitary may be considered the servant of the hypothalamus and even higher structures of the central nervous system (CNS). Several electrophysiological and neuropharmacological studies have been undertaken in the search for the cerebral origins of puberty. This work will help in ascertaining the causes of delayed pubertal onset, a disorder that engenders great consternation among children, their parents and their doctors. Similar neuroendocrinological studies should shed light on what may prove to be a group of primary deficiencies of hypothalamic releasing factors, e.g., thyrotropin releasing hormone (TRH) and growth hormone releasing factor (GHRF) which cause puzzling cases of cretinism and dwarfism, respectively.

3. Developmental Human Genetics - Genetics research within the DBN Branch focuses on studies of gene organization, regulation of gene action during development, mechanisms underlying normal mammalian differentiation, and the developmental basis for genetic defects. These investigations include basic biological approaches as well as therapeutic and familial studies.

The skeletal dysplasias are being studied by one team of DBN-funded scientists. Although many clinical forms of these disorders have been described, little is known of their causes. This research team is investigating the clinical, genetic, morphologic and biochemical characteristics of each of the skeletal dysplasias and has studied over 200 dwarfed individuals this year. An International Referral Center has been established resulting in receipt of clinical, radiographic and biopsy material from 120 patients with skeletal dysplasias around the world.

Another group of investigators is evaluating a group of children with sex chromosomal abnormalities. Preliminary data indicate that the general outlook is good for this group. This study may remove the stigma of increased risk for intellectual, emotional, social, and physical problems in later life. The study sample is now entering adolescence and will be closely observed during this developmental stage. The final data will be especially important due to increased use of amniocentesis for prenatal diagnosis and concomitant increases in diagnosis of sex chromosomal abnormalities.

In February, 1979, DBN staff attended a Public Hearing on alphafetoprotein which was organized by the Food and Drug Administration (FDA). This meeting is relevant to the Consensus Development Conference on Antenatal Diagnosis that was organized by NICHD and took place in March, 1979. Many of the same professional and consumer organization representatives participated in both meetings.

NICHD co-sponsored a National Cystic Fibrosis Conference in March, 1979, along with the Cystic Fibrosis Foundation, NHLBI, NIAMDD, and the NIAID. Since cystic fibrosis (CF) is considered a disease in search of an idea, basic scientists, not currently involved in CF research made up a major portion of the participants. Recommendations of this meeting are to be published. Dr. Dayton represents the NICHD on the NIH Cystic Fibrosis Coordinating Committee and was involved in the development of a Program Announcement of research interests in CF. This appeared in the NIH Guide for Grants and Contracts and was jointly sponsored by the other Institutes represented on the NIH Cystic Fibrosis Coordinating Committee.

4. Developmental Nutrition - The DBN Branch encourages research directed toward understanding the relationship of nutrition to human developmental processes. The overall emphasis of the program is to examine nutrition as a potentiating factor in normal development. This program encompasses studies of nutrition throughout the human developmental period, beginning with nutritional factors relating to pregnancy and fetal development and proceeding through neonatal nutritional adaptation to problems of nutrition and growth during childhood and adolescence.

The Branch supports many studies of the complex relationship that exists between the mother and her fetus and the means by which nutrients pass from one to the other. Some of this research focuses on the prediction of fetal status by the examination of various factors in the mother's blood and in amniotic fluid. Other investigators focus on the mechanisms of placental transfer of essential nutrients. Research is also supported that is designed to ascertain the effects of excessive or deficient amounts of certain nutrients on the morphologic and endocrinologic development of the fetus.

More research is planned on intrauterine growth retardation (IUGR). Emphasis will be placed on developing predictors of IUGR in its early stages, assessing the mental development of infants in this category, and exploring appropriate nutritional intervention that will potentiate their mental and physical development. Such intervention programs will eventually be directed toward maternal nutrition, direct fetal nutritional therapy, and post-natal nutrition that will minimize developmental deficits resulting from IUGR. A study that should contribute to resolving the question of providing nutritional therapy in utero is currently supported by the Branch. The purpose of this project is to evaluate the extent to which nutrients can be supplied to the fetus via its gastrointestinal tract. It is based on the observation that the fetal lamb swallows up to 500 ml of amniotic fluid daily. Success with this model system has two potentially exciting outcomes. Poor nutrition in utero is believed to lead to the development of intrauterine growth retardation. These studies suggest that when placental transfer of nutrients is decreased, gastrointestinal supplementation of the fetus may be possible. In addition, the potential for

advancing the development of the gastrointestinal tract by stimulation with nutrients has implications for feeding of premature infants in intensive care nurseries.

Assessment of nutritional status presents a major problem of research and development. The Branch is now funding a promising project in this area. An innovative procedure is being developed for assessment of iron status by measuring serum ferritin concentrations. An enzyme-immunoassay procedure, has been developed which eliminates the hazards of ^{125}Iodine and makes possible the application of this sophisticated technique to routine clinical evaluations.

5. Childhood Obesity and Nutritional Antecedents of Adult Disease - The major objective of this program is to advance our knowledge of the precursors and determinants of obesity. A consortium of seven longitudinal studies has been assembled in order to examine the roles played by dietary factors, heredity, early growth rates, and childhood illnesses in the production of obesity. In this large study central issues under examination are: the natural history of obesity; the importance of heredity and events in early life in the production of obesity; the relative importance of fat cell populations in this relationship; and the contribution of obesity to the overall risk of cardiovascular disease later in life. Research work on predicting outcome of behavioral modification programs for weight reduction are also under way, and this avenue of research will be expanded.

Basic research studies on adipocyte populations in normal and obese infants, children and adolescents are also under way. Enzymatic markers of preadipocytes are being developed, and the metabolism and proliferation of adipocytes are being studied in detail.

Present plans and program initiatives emphasize research on the cerebral control of food intake, including work on: cerebral processing of internal signals of hunger and satiety; external cues in the environment that trigger eating behavior; stimulus sensitivity of obese and lean subjects; and the use and effectiveness of behavior modification in encouraging good nutritional habits and eliminating deleterious ones.

In November, 1978, staff of the DBN Branch collaborated with staff from five other Institutes to write a Program Announcement entitled Studies on Overnutrition and Obesity. The first applications received in response to this announcement have been evaluated by initial review groups and will be considered by the Council in October. In June, 1979, a workshop co-sponsored by the NICHD and the National Institute on Aging (NIA) was held on Nutrition, Behavior and the Life Cycle. The cerebral control of food intake, analysis of feeding patterns, and other factors present during infancy and childhood which may contribute to the development of obesity in adulthood were discussed. A Program Announcement to be drafted by staff of the NIA and the NICHD will focus on these aspects of behavior and nutrition.

6. Developmental Immunology - Studies of the development of the human immunologic system are an essential aspect of the NICHD research emphasis on prevention and treatment of diseases associated with reproduction, pregnancy, infancy, and childhood. Cell differentiation from primitive precursors is vital

to the function of this system and dictates a need for strong alignment with cell biology. Application of this information to specific health problems requires support of clinical approaches. In addition, biomedical observations and epidemiological data have demonstrated that certain diseases and disorders are often expressed in highest incidence within specific age clusters during development. Understanding of the maturation of body defenses over time will lead to an understanding of the mechanisms which subject specific age groups to increased vulnerability.

An important example of such a health research program is the intrauterine development of the mammalian fetus containing paternal genetic characteristics foreign to the mother. This can better be understood by study of the mechanisms which maintain the fetus in close association with mother for a period of time markedly exceeding the normal rejection time of most allografts. Defense mechanisms combatting intrauterine infection must also be understood in order to decrease morbidity associated with pregnancy.

Other studies are being conducted in the perinatal period in order to identify children at risk for later development of allergy. High risk groups are identified on the basis of history and symptoms in parents. Studies of HL-A types, IgE levels and other immunologic parameters are investigated in amniotic fluid, in cord blood, and in serial specimens from infants and parents.

Another example of such immunologic vulnerability involves increased morbidity and mortality in premature and newborn babies caused by gram negative bacilli and Group B streptococci. Publication of the proceedings of an NICHD-sponsored conference entitled Development of Host Defenses appeared in print within the past year and represents the forefront of current thinking in the field of human defense. It emphasizes the ontogeny of human immunity, maternal relationships with the developing fetus, and immunologic responses in persistent intrauterine infections.

A new research area being developed by the DBN Branch concerns immunologic factors in breast milk as a source of newborn immunity. Epidemiologic studies suggest that newborn infants who are breast-fed have better protection against enteric infections than bottle-fed controls. To stimulate further interest in this research area, a conference was held in September, 1978, entitled, "The Immunology of Breast Milk." Highlighted areas included the humoral and cellular immunologic factors of breast milk, their protective potential, and the maternal and newborn mechanisms involved. The proceedings of this conference will be published in October, 1979.

DBN staff represents NICHD on the NIH Coordinating Committee for Blood-Related Activities. The committee plans to assist in developing a stronger NIH-wide program for all programs of blood-related research. Better cooperation and information-exchange for blood-related research at NIH should result. Ontogenetic development and differentiation of white blood cells is such a research area and is important for the NICHD developmental immunology program.

7. Infant Nutrition - The Branch supports a program of research on infant feeding. Clinical difficulties associated with the nutritional management of infants born prematurely and of infants who fail to attain normal rates of growth

in utero are among the most important research problems of this area. Two such problems lie at the heart of the issue of nutrition of low-birth-weight infants. The primary problem involves determining the amount of protein in the diet that will provide optimal nutrition for these infants. The second problem is ascertaining what type of protein (human milk protein versus cow milk protein) will allow growth of these small infants.

This problem and similar problems involving other nutrients can best be resolved by a unified consideration of the entire issue of infant nutrition. Two closely related avenues of investigation must be pursued: The first is to re-examine human breast milk by modern techniques; the second is to expand the detailed understanding of human metabolic development.

One facet of this program is a contract effort entitled, "Evaluation of Breast Milk for Banking." This project aims to fill an important gap in research on infant nutrition by developing guidelines for collection, storage, processing, and distribution of human milk. Advances in this technology are pivotal to the increased use of human milk for feeding premature infants. The focus of this project is to develop technologies that minimize the loss or inactivation of important nutritional and immunologic properties; such losses are known to occur during conventional processing procedures. In future years this contract effort will expand the analysis of nutritional variability of colostrum and milk. Eventually contract proposals may be sought to isolate, characterize, and maintain fractions of colostrum and milk that are of biomedical interest and of potential therapeutic use. The purpose of this effort would be to supply clinical and basic researchers with fluid fractions that may serve to meet more precisely the nutritional needs of the neonate.

8. Cultural and Behavioral Aspects of Nutrition - The objective of this new program is to gain understanding of the roles played by behavioral aspects of nutrition in the prevention of disease and in the promotion of health. The new program supports studies of behavioral, social and cultural influences on diet and nutrition.

Nutritional Individuality: Research is being encouraged on genetic-environmental interactions that determine nutritional individuality as well as on cultural and behavioral determinants of individual nutrient intake, hunger, and satiety. Studies of dietary choices and research on the anthropologic basis of food totemism and taboos are emphasized, as well as investigations on psychological, genetic, and chemical determinants of taste and the development of dietary patterns.

The staff of the Branch recently developed three Requests for Proposals (RFPs) for contracts in the area of nutritional individuality. These RFPs were published in the NIH Guide for Grants and Contracts in January and February of this year. At the time of this writing twenty-six proposals are being reviewed.

The first RFP sought epidemiological studies of nutritional individuality, e.g., soya intolerance, among various ethnic and racial groups. The second RFP sought mechanistic studies of genetic and biochemical explanations for various kinds of nutritional individuality and idiosyncratic metabolism of certain foods. The third RFP sought clinical studies of diets that produce intolerance and of diets

that can prevent or ameliorate clinically significant food intolerances.

Nutrition and Evolution: Much needs to be learned about the nutritional needs of individuals and of ethnic and cultural groups. More study is needed of the evolutionary modification of digestive processes under the selective pressure of certain foods as well as of the relationship of the geophysical environment to cuisine, culture, health and disease.

There is also a need to determine ethnic intolerance to certain foods, drugs, and other ingested substances. Biochemical divergence rather than similarity should be expected when comparing peoples who have been long separated geographically or culturally.

Development and Modification of Dietary Patterns: In addition to evolutionary and developmental problems in nutrition, studies are needed on other aspects of nutrition, such as development of taste preferences and eating habits; modification of poor dietary habits; adaptation to dietary change; and evaluation of success in long-term dietary modification.

Pregnancy: It has been shown that in situations where prenatal counseling and care are similar, there is a wide difference in weight gain during pregnancy. Research is needed to identify the nutritional, behavioral, and physiological factors that underlie these differences, particularly in regard to the pregnant adolescent. Pica, e.g., starch- and clay-eating, is common among pregnant women in certain ethnic and racial groups. Other food cravings and aversions occur frequently during pregnancy. The social, psychological and physiological determinants of such behavior must be better understood, especially in reference to the effects of aberrant patterns of food consumption and maternal and fetal health.

As indicated previously, in June the NIA and the NICHD sponsored a workshop on Behavior, Nutrition and the Life Cycle. The staffs of these two Institutes are now drafting a Program Announcement seeking applications in these areas of research.

9. Developmental Gastroenterology: In order for a substance to have a functional role in development it must first reach the site of metabolic activity. The initial barriers to this process are the digestive and absorptive capacities of the developing gastrointestinal tract. The gastrointestinal tract is the primary interface between the developing infant and its nutritional environment. If, because of disease, congenital defects, or extraordinary metabolic demands, the function of the gastrointestinal tract is impaired, then physiological development will suffer. There currently exists little information concerning these critical factors in efficacious nutrition during the developmental period.

An important issue which ties several of these considerations together concerns the effects of human milk on the growth and maturation of gastrointestinal mucosa. The Branch already supports research on growth factors; in this aspect of the Nutrition program special efforts will be made to define growth factors in human milk. Studies on the biliary tract are also in progress that seek to define changes in bile salts associated with various nutritional intakes (human milk, cow milk, soy-bean based formula, etc.).

The Request for Applications on Infant Nutrition stimulated several new and exciting studies in the area of developmental gastroenterology. Additional development of this important field was achieved through a recent conference entitled "Gastrointestinal Development: Current Status and Future Prospects for Research." This conference served to bring together several related aspects of gastrointestinal research which had not previously been associated. Important topics were discussed at this seminal conference which point the way toward new programmatic goals. They included: Development of screening tests for celiac disease. There are many individuals who are gluten-sensitive but whose illness remains subclinical. The only indication of gluten sensitivity may be a low hemoglobin, mild hypocalcemia, low-grade fever or unexplained joint pains after eating foods that contain gluten. Diagnosis and dietary treatment of these individuals would be a major step in reducing the chronic morbidity of this population.

Development of Gut Defenses: The majority of newborn infections are believed to gain entry to the body through the gastrointestinal tract. Active immunologic cells and humoral substances appear in the gut, develop protective functions, and normally prevent entry of infectious organisms as well as of other foreign proteins to block the development of infections, food allergies and gastrointestinal diseases. It will be important to learn how gut defenses develop during early life and how they remain competent to prevent illness and disease.

Enteral Nutritional Therapy: Surprisingly, a rested gut deprived of food does not heal as rapidly as one in constant use. It has recently been shown that enteral feeding of patients with infantile diarrhea shortened hospitalization time by one-third when compared with parenteral nutrient therapy. Clinical trials are now needed to establish therapeutic guidelines for the use of enteral nutrition in cases of infantile diarrhea and other diseases of the gut.

10. Malnutrition and the Immune Response - For several years the NICHD has supported studies concerned with the effect of malnutrition on the developing immune system in young children. Malnutrition and infection are major causes of mortality and morbidity in children within the U.S.A. as well as in developing countries throughout the world. NICHD-supported studies have documented that immunologic alterations occur in malnourished children. Although antibody production and ability of white blood cells to ingest and kill bacteria appear intact in malnourished populations, a defect in cell-mediated immunity has been found.

One group of NICHD investigators has noted that there are significant individual differences in immunologic response of children despite similar degrees of malnutrition. It was also found that in most infants the immunologic defects were rapidly reversible upon institution of an adequate diet. However, some children did not recover rapidly despite adequate nutritional therapy and had long infectious episodes or died. Therefore, studies were initiated to evaluate immunologic defenses in relation to intrauterine development since preliminary data indicated that intrauterine malnutrition might be implicated. These studies have shown that cell-mediated immunity is reduced in the intrauterine-growth-retarded infant and that the problem is still present at 12 months of age, perhaps due to slow recovery or the presence of postnatal protein-calorie mal-

nutrition. Further evaluation of these data are planned.

The biological preparation of a foreign substance for ingestion and ultimate destruction by white blood cells is known as opsonization. Another research team is evaluating opsonization functions in malnourished children. It involves an antigen's being coated with its specific antibody. These investigators have found decreased opsonizing abilities in malnourished children are are carrying on clinical trials to evaluate various therapeutic measures to correct this deficiency. Since malnutrition is a severe problem in many areas of the world, these studies have the potential for widespread application to health problems.

Research Training

The Branch provides two types of training awards: individual fellowships (F32s) and institutional training awards (T32s). During its existence from FY 77 to FY 79, the DBN Branch supported annually approximately 20 individual fellowships (F32) and 10 institutional awards (T32).

The DBN Branch sponsors research training in support of its regular research programs. Awards are made for basic and clinical research training in the areas of Developmental Biology, Pediatric Endocrinology, Developmental Immunology, and Nutrition. Training in these fields emphasizes study of those events, both normal and abnormal that affect growth and development.

Research training awards represent a rather small fraction of the projects supported by the Branch. In all training categories combined (T32 and F32) there are currently 30 active grants that amount to $1.6 million. This represents approximately seven percent of the projected Branch budget of $22.7 million for FY 79.

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October 1, 1978 through September 30, 1979
Developmental Biology and Nutrition Branch
Contract and Collaborative Research

Contract Title : Genetic Linkage Analysis of Juvenile Diabetes
Contract No. : NO1-HD-8-2946
Contractor : Regents of the University of Minnesota, Minneapolis, Minn.
Jose Barbosa, M.D.
Money Allocated: FY 79: None as of this writing
FY 78: $194,542

The principal goal of this contract effort is to disclose genetic associations between the juvenile diabetic phenotype and various polymorphic marker alleles that segregate within families of patients with juvenile diabetes. Certain marker alleles at the B and D loci of the HLA system on human chromosome 6 and perhaps at B(f) (Properidin) locus on chromosome 6 seem to segregate with a diabetogenic gene or genes. Identification of markers could provide measures of risk for individuals who are relatives of insulin-dependent diabetics. Studies of this nature are the first step in the ultimate prevention of insulin-dependent diabetes. Detailed knowledge of the genotypes involved may allow for identification of environmental factors that interact with mutant genes to trigger the disease. One would then be in a better position to protect susceptible individuals from potential environmental insults.

This contract is a comprehensive study of families containing one or more members with insulin-dependent diabetes mellitus (IDDM). Subjects are tested for 220 genetic markers and several biochemical and histopathological indicators. At this writing many of these parameters have been determined in 398 subjects from 80 diabetic families.

During the first year the contractor has ascertained nearly 400 IDDM probands. The ascertainment is ahead of schedule and will probably go beyond the originally estimated 500 IDDM patients by September 30, 1979.

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October 1, 1978 through September 30, 1979
Developmental Biology and Nutrition Branch
Contract and Collaborative Research

Contract Title : Human Milk Bank Research and Distribution Center
Contract No. : NO1-HD-8-2828
Contractor : Baylor College of Medicine, Houston, Texas
Buford Nichols, M.D.
Money Allocated: FY 79: None as of this writing
FY 78: $260,529

This contract effort is designed to resolve major problems inherent in the collection, storage, distribution and utilization of banked human milk and colostrum in clinical settings. This is a phased operation in which methods of collection and storage are first being studied systematically. In the second phase the variability of human milk and colostrum will be studied in relation to maternal nutritional status, feeding frequency and other factors. In the third phase the Milk Bank will serve the medical research community by providing well-documented samples of human milk and colostrum and fractions thereof for basic and clinical research. This contract is necessary to place the therapeutic uses of human milk and colostrum on a firm footing.

The workshop for the first year of this contract included evaluation of techniques for collecting human milk, evaluation of various storage containers, evaluation of storage temperatures, evaluation of maternal age and duration of lactation and evaluation of various processing technologies.

Hand expression and mechanical pump expression were compared in terms of the volume of milk obtained with each, the fat content of milk obtained with each and the extent of bacterial contamination of milk obtained with each. In comparing these collection techniques valuable information has been obtained concerning donor acceptance of each collection method. General information concerning the details of recruiting donors, the expectations of donors and other important aspects of donor reactions were gathered as well.

The studies of the effects of freezing on the physical characteristics of the caseins of both colostrum and mature milk have been completed. In addition, considerable data have been obtained concerning the lipase activity of both colostrum and mature milk and the effects of heat treatment on this activity. Data concerning the effects of heat on bacterial counts of human milk have also been obtained.

Important new information provided from work under this contract includes: (1) the demonstration of the wide variability of both nutrient and immunological components of human milk; (2) the lack of an effect of storage container on nutrient composition; (3) the differences between colostrum and mature milk with respect to effects of storage container and duration of storage; (4) the general superiority of pyrex over polyethylene containers with respect to effects on immunological components; (5) the increase in number of both macrophages and lymphocytes during storage in all containers, particularly glass; (6) the relative heat sensitivity of human milk lipase in comparison to cow milk lipase;

(7) the apparent germicidal effect of human milk, in vitro; (8) the ease with which bacterial contamination of human milk can be decreased by heat; and (9) the different physical effects of freezing on caseins of human colostrum and milk.

The progress to date has been satisfactory and the new information obtained is important both with respect to our knowledge concerning the biology of human milk and with respect to human milk banking.

NICHD Annual Report
October 1, 1978 through September 30, 1979
Human Learning and Behavior Branch
Center for Research for Mothers and Children

The Human Learning and Behavior Branch (HLB) is concerned with the development of human behavior from infancy through adolescence to maturity. Through its support of fundamental research and training for research, the HLB Branch seeks to determine how behavioral, biological and social factors interact and influence the normal development of the individual and to identify the factors which may interfer with or prevent normal development. This Branch gives special attention to the human communicative, learning and cognitive processes especially, speech and language, and the development of the ability to read as well as those factors which may contribute to abnormalities or disorders in these important human skills. The disorder, developmental dyslexia, has received special research attention.

Attention has also been given to personality and social development, early parent-child interactions and the study of families. A major effort has been made to investigate the biological, environmental, and social factors which may lead children to behavior that is harmful to their health.

As a direct consequence of this special interest the HLB Branch has assumed a major responsibility for a significant portion of the NICHD's contribution to the Secretary's (HEW) smoking initative. As is described later in this report the Branch has encouraged and directed scientists throughout this country to investigate the biological, personality, social and other environmental reasons for children and adolescents to initiate cigarette smoking.

During a previous reporting period the HLB Branch published a request for applications (RFA) for research grant proposals in this area. During the past year a request for proposals for research under contract on childhood conditions that lead to habitual cigarette smoking was issued. It is anticipated that several projects will be funded through this support mechanism. More details on this activity will follow in a subsequent section of this report.

During this reporting period staff of the HLB Branch served as co-editors of the proceedings of two conferences supported by the Branch. The conference, Child Phonology: Production, Perception and Deviation was edited and submitted to the Academic Press under the title, Child Phonology: Perception and Production. An early spring, 1980 publication is anticipated. The cross-language conference on Orthography, Reading and Dyslexia held in September, 1978 was submitted to the University Park Press. A December 1979 publication date has been promised.

Training for Research

The HLB Branch supports two kinds of research training activities, training grants to institutions and postdoctoral fellowships to individuals. The training grants provide support for both predoctoral and postdoctoral students who are selected by the training grant staff. The postdoctoral fellowship applications are reviewed and awarded to the individual applicant in a manner similar to research grants.

During FY '79 seven training grants and 18 individual postdoctoral fellowships were supported by the HLB Branch.

Human Communication

The HLB Branch supports studies that are concerned with the biomedical and behavioral processes and mechanisms involved in the development of normal communicative skills and those factors which may interfer with normal development of these skills. The role that communication plays in human learning, growth and development is also an area of interest. In recent years the major emphasis has been on studies of the acquisition and development in children of speech, language and reading with a special interest in the reading disorder, developmental dyslexia.

Developmental dyslexia, often referred to as a specific reading disability or a specific learning disorder, is a condition in which a child's reading skills are significantly below expectation. Such children apparently have normal sensory processes, normal speech, perform within normal limits on tests of intelligence and have received customary reading instructions. These unexpected reading failures are a serious social, academic and employment problem for the individual. It has been estimated that 10 to 30 percent of the total grade school population have reading skills significantly below expectation for their chronological and mental age.

Although the HLB Branch has supported many individual research grants and activities related to reading and dyslexia, it was felt that a major research program in this area was needed and that such a program could produce significant results. HLB Staff, therefore, made a concerted effort to identify and stimulate interdisciplinary research groups to develop research applications in this area. Following several years of this "programming" and review, the Branch awarded a large grant to the Rutgers Medical School for support of a "center for neurocognitive study of dyslexia." This research team draws on researchers from the fields of psychology, linguistics, education, neurology and medicine.

The research group is using a multi-step approach: (1) component parts of the reading tasks are being isolated, (2) the performance of dyslexics, normal and learning disabled non-dyslexic children are being compared on those components of the task which are most likely to cause difficulty for the dyslexic, (3) when the dyslexic is found to be deficient with respect to the two groups, the group is attempting to determine the course of the difficulty. Because dyslexia has different manifestations and possibly different processes at different ages, this research team is examining persons from preschool to adulthood.

One of the many strengths of this research team is the large number of established researchers on their staff who have a history of concern for reading problems. Moreover, the team includes a number of young and promising investigators who can be expected to influence research on this topic for many years.

Another important strength in this program is that excellent cooperation has been established not only with individual schools but with the entire school

district. The extent of this collaboration with so many different schools is unusual and attests to the sensitivity of the Rutgers research group in working with school personnel, parents and their children. The HLB Branch expects important research findings to come from this outstanding team of experts.

Another research team which has received programmatic support from the HLB Branch is studying the nature of the speech code (language by ear) and the reading process (language by eye). These two seemingly different concepts are intimately related, hence very appropriate for a single research effort not only because they both deal with language, but also because they are both concerned with much of the same part of it, namely, the phonetic, and more abstractly, phonologic structure. In the first part the emphasis is on the relation between the acoustic signal and the phonetic structure it encodes, and with the processes by which a listener goes from one to the other. In the second part the investigators have taken into account the fact that alphabetic orthographies like that of English transcribe the phonologic structure of the language, of which the phonetic structures of the first section are a somewhat less abstract reflection. The effort here is to find out whether, how much and for what purposes readers use the phonologic and phonetic information that the orthography makes available to them.

Whereas, for many years research in the area of speech perception was conducted by scientists exclusively interested in oral communication, while at the same time reading research was being conducted by independent investigators solely interested in visual aspects of language, this research team has integrated both forms of language reception.

Moreover, especially in linguistic circles, there has been an extended conflict of opinions. Some scholars have concluded that speech-related processes contribute positively and significantly to reading in all its progressions up to and including fluency, while there are those who would conclude that the role of speech-related processes is trivial at best, negative at most, and rarely endures beyond the beginning stages of learning to read.

The grantees of this New Haven, Connecticut based research team believe these conflicts reflect as much confusion as substantive disagreement. With HLB Branch support their research has lead them to recognize that any evaluation of the contribution of speech to the how of reading must be in reference to who the reader is and to what it is that is being read. The team has focused on certain processes derived from speech that might mediate reading in distinctively different ways and they are looking at these processes across variations in readers and variations in what is being read.

Learning and Cognitive Development

The HLB Branch actively encourages research in learning, that is, the process by which individuals acquire, extinguish, or modify knowledge, skills, and habits, and with relevance to both biological and behavioral determinants. Within the broad areas of attention, perception, and sensory processes, the Branch supports developmental studies of conditioning, discrimination, memory, imagery, object permanence, and concept formation. Other grantees supported by the Branch are investigating how biological, personality and social factors

influence cognitive development. One study is relating the production of sex hormones in utero to such subsequent behavioral characteristics as aggressiveness and timidity, and cognitive skills in the infant and young child. Several investigators are also exploring how cognitive ability is affected by temperament and such self-regulatory processes as resistance to temptation, delay of gratification and goal-directed effort.

Basic and primitive processes in learning and cognition can best be studied by experimental manipulation of animal development in order to identify the critical periods at which certain behaviors appear or fail to appear and are often accompanied by neurological changes. One HLB grantee is studying how prenatal sensory and perceptual experiences affect subsequent behavior in young ducklings. Studies of imprinting have shown that female nesting ducks give a species characteristic call as their young emerge from the shell and later give the same call when they lead the young from the nest; ducks hatched in isolation will still follow the call, and even duck embryos (several days before hatching) will respond to the call by bill clapping. However, if embryos are prevented from this self stimulation of bill clapping they fail to differentiate their own species call from that of another species for a period of 48 hours after hatching. Further studies seek to determine exactly how embryonic experience (bill clapping) affects the ducklings later ability to discriminate their own species call from that of another.

Studies of early infancy are important for determining the infant's level of perceptual competence at birth and the subsequent progress resulting from both maturation and experience. At the neonate level, one grantee has examined such behavioral characteristics as sucking activity, heart rate, breathing, and eye movements in order to develop a set of conditioning procedures which can then be used to accurately assess memory and perceptual preferences in the newborn. Research on visual perception in infancy receives strong support from the Branch because of the complexity of visual processes and the primacy of vision through the human life span. In this area, NICHD-supported studies revealed that the infant's capacity to differentiate between different visual stimuli is very impressive. For example, three-month-old infants can recognize the difference between high contrasting photographs and simple figures which differ in both form and color. By four months they can distinguish between fabric swatches varying in color and pattern, red and yellow figures varying in shape only, black and white patterns of circular form and in simple rotating forms.

Pattern perception is being studied by several grantees. It is now well documented that newborns prefer large elements over small ones, but with increasing age they also prefer more elements, that is, complex stimuli rather than single elements, probably due to neural motivation.

One grantee, supported by the HLB Branch, is studying the early perceptual patterning of term, preterm and exceptional infants from 3-23 weeks of age. Preferences for visual patterns of varying degrees of complexity have been determined by such measures as visual fixation time, eye movements visually-evoked cortical potentials, and other psychophysiological indicators. Thus far, preliminary data analysis indicates that already at birth the infant looks selectively at a variety of aspects of patterning, indicative of a

substantial unlearned neurobiological basis for pattern perception. For infant-rearing practices, these results further emphasize the importance of providing many and varied early opportunities in the home for the infant to visually explore and learn about objects, people, and places.

Further research with pre-term infants seeks to determine the extent of their visual abilities; in a practical sense such findings can help clinical staff provide visual stimulation to those infants who must remain in the hospital for prolonged periods of time. Similar studies with neurologically high-risk infants will help differentiate infants with less serious peripheral deficiences that may disappear or be remedied in time from those with more critical central-cortical damage. These longitudinal studies of young infants may also reveal additional developmental indicators of retardation in perception and attention that could be of use in early prediction and diagnosis, thus allowing possible treatment or special care for such children.

In another study the visual responses of young infants to contour and configuration are being investigated in the first few months of age to determine the effect of early experience upon visual orientation. Premature infants are being compared with full term infants to see if visual processing deficits are associated with prematurity.

Perception is just one component of cognitive development; attention, memory, discrimination learning, problem solving and intelligence are other factors. The development of memory is being studied throughout the life cycle: in terms of habituation (decreased level of response) to visual stimuli in infants, the development of children's mnemonic skills; semantic memory in preschool, that is the process by which children remember meaningful pictures in recognition tasks; and the development of metamemory (knowledge about memory) in elementary and high school students, specifically how children devise strategies for comprehending various memory tasks. In addition, one grantee is studying adult long-term, memory, in some cases over a period of 50 years or more. Using a cross-sectional approach, the investigator has contacted college alumni of widely varying ages who are then shown pictures of classmates, buildings and maps and asked to identify them. Subject and situational factors are controlled by statistical techniques. Family resemblances in memory and psychomotor tasks is another subject being investigated with Branch support.

The memory capacities of children with dyslexia also receives Branch support. Although it has been argued that such children have problems in perceiving various visual stimuli because of poor memory, the research findings actually indicate that those dyslexic children who were high on verbal IQ but low in performance IQ performed even better than normal readers in recalling words presented auditorially, whereas those with low verbal IQ and high performance IQ scored the same as normal readers. The results of this study suggest that there are different types of dyslexias and children diagnosed as such are not a homogeneous group.

The development of higher cognitive processes in children and adolescents has attracted other NICHD supported investigators. One grantee is examining the hierarchical development of motivation in children at various age levels with tasks ranging from simple two-color discrimination tests to more complex

puzzle activities. In addition to assessing the children's ability to master such tasks, the investigator is also looking at Piagetian and other cognitive and achievement measures. An earlier finding that girls show less motivation to master tasks and a greater need for approval in certain types of problem solving situations is being replicated, and other sex role variables are being examined.

Anticipatory imagery as it relates to intellectual development in elementary school-aged children is the focus of still another Branch supported study. Given a series of geometric and sequential movement tasks, the children are asked to produce premises and make inferences; the findings show the process by which imagery develops with age.

One NICHD grantee is studying the developmental process by which children and adults acquire orientation to their spatial surroundings and learn to recognize routes and destinations. In a series of simulated journeys subjects try to reconstruct the sequence of specified landmarks along the route. So-called "cognitive maps", internal representations of the environment, may serve to guide the individuals in finding their way.

Conclusion: During the past fiscal year the area of learning, specific in learning disabilities, accounted for 32 research grants, 3 program projects, 1 research career award, 2 training grants, 6 fellowships and 2 contracts. This represents 42 percent in number and 40 percent in dollars of HLB's research activities.

Personality and Social Development

Cigarette Smoking: The Secretary's initiative in the prevention of smoking, highlighted by the 1979 Surgeon General's Report on Smoking and Health, has greatly encouraged research efforts to identify the antecedent conditions that lead children to take up cigarette smoking and other harmful health habits, such as overeating, lack of exercise, or various risk-taking behaviors. At the same time investigators are also being asked to study how good health habits and lifestyles develop in children. A request for grant applications (RFA), entitled "Health Promotion & Prevention of Smoking and other Behaviors Detrimental to Health" brought forth a total of 93 research grant applications for the two review cycles ending in the January and May Advisory Councils: 28 applications were approved. About 80 percent of the applications submitted were behavioral, 20 percent biomedical. Of the approved applications, 12 received sufficiently good priority scores to be funded in FY 79; with one exception, all are behavioral. The majority of studies focus on issues related to the initiation of smoking, often with interventions to teach teenagers strategies of resisting peers, family, and environmental pressures to take up smoking or other harmful health habits. One innovative study is exploring the health beliefs and practices of parents of preschool children, from which the investigator will design a health curricula for the children in the areas of smoking, nutrition and safety, using such teaching methods as videotapes, puppets, records, role playing, trips, etc. At the same time parallel educational experiences will be conducted with the parents.

Another HLB grantee, who has access to longitudinal data collected on 17,000 children born in Britain during one week in 1970, will study the effect of maternal cigarette smoking during pregnancy on the child's subsequent development (somatic growth, mental ability and achievement) at birth, and at 5 and 10 years of age, allowing for class and educational differences.

A request for contract proposals (RFP), "Childhood Antecedents of Habitual Cigarette Smoking." issued in February, focuses on the antecedent factors which are responsible for the establishment of smoking, rather than the initiation of smoking, as was the case with the RFA. Many, if not most children "experiment" with smoking, but by the age of seventeen, 25 percent of American youth are established (habituated) smokers. The antecedent conditions (both psychological and environmental) that determine whether an adolescent becomes or does not become, an habitual smoker are not well understood. Furthermore, available evidence indicates that: (a) behavior associated with habitual smoking is determined by multiple and interacting conditions and (b) these conditions are likely to differ for youths of different populations and for those in diverse kinds of life circumstances. Prospective offerors were asked to develop a research strategy to identify, within well-defined population groups, the concurrent and interacting conditions that lead to continued non-smoking among adolescents; the study will cover 30 months in all. For the May 1 deadline, 21 proposals were received, and five were judged acceptable. Awards were made to 2 contractors who initiated their studies in September.

Other Research: While current funds for personality and social development amount to less than 11 percent of the total FY79 budget for the HLB Branch, the variety of studies supported is impressive. Studies include personality characteristics, the emergence of social competiency in premature or full-term infants, early pro-social behavior in children, and several significant studies of parent-offspring relationships (primates and humans). The behavioral aspects of children's health and illness receives Branch support, not only in relation to the smoking initiative, but in terms of the effect of the chronically ill child in the family, and the relationship of children's health beliefs and behaviors to those of their parents.

Under a research career development award an HLB Branch grantee has been studying how patients comply with medical recommendations. Findings from several studies indicate that mothers who perceive their children (and themselves) as being in poor health and relatively susceptible to illness are less likely to seek preventive care but more likely to visit physicians for acute illnesses. These and other results have been derived from the Health Belief Model (HBM), which the principal investigator and his associates have developed and evaluated in terms of its ability to predict mothers' behavior in terms of their utilization of pediatric ambulatory care services, their adherence to a drug regime for their asthmatic children, and adherence to a diet prescribed for their obese children.

The family and socialization processes continue to be major research areas. In a broader context, a series of three workshops, funded by the HLB Branch, is examining the family as a biological and social entity. Eminent scholars in the medical and behavioral sciences are attempting to assess the long-term effects of twentieth-century technology (hospital deliveries, Caesarian sections, short-term bottle feeding, etc.), and social change on the family's stability.

Drawing upon evolutionary, comparative, and developmental evidence, the workshop participants are focusing on the biological aspects of contemporary human social behavior with the ultimate aim of identifying research areas that need further study.

Parent-child behavior continues to attract researchers. Animal studies supported by the Branch have focused on maternal behavior in primates in an effort to identify the critical factors in establishing social bonding between the mother and offspring. One grantee is comparing the responses of young monkeys reared with inanimate surrogate mothers to those reared with natural mothers to see if they differ in their ability to recognize the "mother" in a maze-type situation, and another investigator is studying the role of olfaction in the development of the infant monkey's recognition of, and attachment to either its maternal or surrogate mother.

In human studies, one question being asked is: To what extent is the interaction pattern of mother and premature infant different from that of the mother and full-term infant, and do distinct patterns emerge such that one can distinguish early those children who will become socially competent ("social stars") in contrast to those who will not. Two groups of infants, prematures and full-term, are being studied with their mothers for the first four years; both come from lower-income families. The children's development and behavior will be assessed by developmental and neurological tests as well as by home observations.

The impact of Caesarian section delivery on the whole spectrum of relationships of new parents to one another and to their infant during the first year of life is the subject of a recently funded study. The investigator will compare the parenting experiences of infants born by Caesarian section with those born vaginally. The data, obtained by interviews and observations, will include demographic variables, relationships with spouse, family and friends, attitudes toward sex roles and marriage, retrospective views of the pregnancy and birth experiences, in addition to child development measures and behavior.

Although a large number of currently funded studies focus on the personality and social development of infants, several investigators are studying such developmental issues as altruism in childhood, sex role differentiation in adolescence, and continuity of life experiences in young adults. In the altruism study elementary school-age children, through a series of operant conditioning experiments, receive tokens for charitable behavior to peers, with the emphasis on getting the child's explanation of his own helping behavior and of being helped by others. The investigators have also devised another experiment, based on Hartshorne and May's 1929 study of altruism, to determine whether the helping behavior developed in children 5-12 years by modeling techniques is lasting, durable, and unaffected by age.

Another HLB grantee is asking the question, "How do the experiences of the adolescent alter his or her adult life, particularly in terms of sex role differentiation?" Drawing on data collected by James Coleman on 9,000 school students in 1957-58, this grantee sent subjects a follow-up questionnaire 15 years later. The responses received are now being analyzed to see how adolescent and post high school experiences have contributed to adult sex role differences and personal esteem, including a comparison of women who are solely

homemakers and those who also work outside the home. Some preliminary findings suggest that the degree of satisfaction experienced in the parental role fifteen years afterhigh school was influenced by different antecedent conditions for males and females during or prior to high school. For females, the single most significant factor was the reported closeness of the girl's relationship to each parent. For males, with the exception of the closeness of the father-son relationship, a number of socio-economic variables were positively associated with later parental satisfaction, specifically the educational level attained by the parents, the extent of the mother's working outside the home, and the educational expectations of the youth and his friends.

The post adolescent period is also the focus of another study which is collecting extensive biographical data on a large group of college students. Using factor analysis, the investigators have grouped responses to the biographical inventory which identify clusters of people who are relatively homogeneous with respect to their experiences: 23 male subgroups are being contacted after college graduation to determine whether the original clusterings remain valid.

Conclusion: During the past fiscal year, the area of personality and social development has consisted of 8 research grants, 2 research career awards, 1 fellowship. This represents 11 percent in number and 11 percent in dollars of HLB's research activities.

Developmental Behavioral Biology

The interrelatedness of three programs of major activity--learning and cognitive development, human communication, and personality and social development--are most strongly evident in the area of behavioral biology. Although it has long been recognized that congenital and/or genetic disorders of the central nervous system adversely affect the child's intellective-cognitive and social-emotional development, it has only recently been recognized the the "social-cultural environment" also influences the neurobiology of those behaviors. Specifically, sensory impoverished and stressful environments are detrimental to brain development of young organisms in both their structural and functional characteristics. Since the brain is the organ of behavior, major program emphasis is given to the effects of early experiences upon brain development and behavior.

Methodologies available to study human brain function and behavior, however, are clearly limited. Invasive procedures of the living human brain are clearly not possible. Procedures that can safely be used rely on neuroelectrophysiological or neuroendocrine processes, and behavioral genetics. An example of neuroelectrophysiological methods to study human biological brain processes is the evaluation of electrophysiological activity recorded from surface scalp electrodes. These "signals" from the brain are subjected to rigourous mathematical and statistical analysis which permit some degree of objectivity, reliability and validation of measurement. A major focus of activity utilizing these methodologies is the evaluation of how the two hemispheres of the brain process information from the various sensory modalities and how information is relayed between the two brain hemispheres. Evidence obtained from such studies provide additional support for the phenomena of specialization in the

two brain hemispheres. For example, the left hemisphere is primarily involved in analytical, sequential processes, e.g., language and mathematics; the right hemisphere is primarily involved in holistic, spatial and integrative processes, e.g., pattern perception, music, and the emotional/affective characteristics of perceptual/cognitive experiences.

The neuroendocrine system also influences brain function and vice-versa which provides another biological measurement tool, although indirect, of brain function which can be related to behavior functions and life experiences.

Dr. Seymour Levine, an HLB grantee, has been studying the relationship between certain hormones, behaviors and environments to understand one of the most important issues of survival: the capacity of an organism to adapt to a wide spectrum of environmental stress. One of the most important stress response systems is the pituitary-adrenal axis which has been the focus of Dr. Levine's studies. He has shown that social and physical stress alters adrenocortical activity and that such stresses upon rodent pups induces elevated corticosteroids in the mothers. Malnutrition has also been shown to affect the adrenocortical response to stress and the relationship of these events to the maternal-infant relationship are being systematically evaluated. Particular attention is being given to the relationship of stress and adrenocortical responsivity to maternal-infant attachment behavior.

Behavioral genetics is another methodology which seeks to trace genotypical influences upon behavior as these influences are modulated by environmental factors. For example, Dr. John DeFries, another HLB grantee, is studying the genetic bases for reading disabilities. Available evidence indicates that there is no simple genetic model which can account for the results of various studies on reading disabilities and that various "subtypes" of reading disabilities exists. Monozygotic and dizygotic twin studies, however, have provided some support for a genetic component in reading disabilities. Preliminary findings by Dr. DeFries' group has implicated chromosome #15 in a selected study of reading disabled families. This genetic marker will be systematically evaluated in a larger sample of reading disabled families.

The Developmental Behavioral Biology section of the HLB Branch is balanced in its support of human and animal research and training activities. In the category of human subject studies there are fourteen research projects, two postdoctoral fellowships and one training program. These human studies are being supported at a total cost of $1,023,903. In the category of animal studies there are ten research projects, two fellowships; one research career development grant; and one research project within a Minority Biomedical Support project. These animal studies are being supported at a cost of $736,636. The total support for the Developmental Behavioral Biology portion of the HLB Branch is $1,760,539. Research efforts at the human level include behavioral genetic studies of perceptual and cognitive functions, particularly, as they may relate to the development of learning disorders and reading disabilities.

Quantitative electrophysiological studies of brain function are extremely sophisticated approaches to quantify hemispheric cerebral specialization in the processing of cognitive information. Findings from these studies may

yield clues to understanding disorders of information processing in learning disorders, particularly, reading disabilities. Additional studies are evaluating sensory psychophysiological mechanisms in infants as they relate to auditory competence, short-term memory and vestibular functions during development.

Studies in behavioral nutrition by Dr. Ernesto Pollitt, another HLB grantee, are evaluating the behavioral consequences of deficiency in iron and micronutrients during infancy and childhood. These studies have shown that mild iron deficiency impairs selective attention and memory processes which are reversable through therapy. Additional studies are planned to evaluate whether more severe iron deficiency will result in greater adverse effects upon higher complex cognitive processes.

Animal studies include basic research on innovative quantitative methods in the electrophysiology of brain function. Dr. Don Jewett, an NICHD grantee who discovered the brainstem evoked responses called "Jewett Waves," has made another potentially significant contribution by discovering a method to portray cortical evoked potentials in three dimensions. Prior to this discovery, brain evoked responses were portrayed in only two dimensions (voltage by time). This third dimension-space-adds an entirely new dimension of characterizing information processing in the brain and future systematic studies are needed to determine its ultimate value and usefulness.

Other animal studies include the effect of early deprivation and insults upon the developing nervous system and behavior. Studies upon the consequences of "social isolation" during the formative periods of development or what has been called "maternal-social" deprivation have been shown to result in the failure to develop affectional bonds, aggression and abnormal structural development of the brain. Specifically, Dr. Austin Riesen, another HLB grantee, has shown that dendritic structures in somatosensory and motor cortex but not visual cortex are abnormal in "maternally socially" deprived animals. These findings may have substantial social and emotional health implications for how human infants and children are reared since sensory deprivation during the formative periods of brain development can lead to abnormal brain development and behavior. The isolation of premature newborns in health care and other infant/child rearing practices often involve low levels of somatosensory and vestibular stimulation (touching and handling) which places such newborns and infants "at risk" for abnormal brain development because of the sensory deprivation characteristics of these environments.

The failure to develop affectional bonds in the mother-infant and father-child relationship is one of the most important social/emotional health problems of our times. The failure of these early developmental experiences of affectional bonding has been linked to a variety of disturbances in social, emotional and intellectual/cognitive functioning. There is a paucity of information and understanding of the neurobiological substrates and processes that mediate affectional bonding. Major efforts are urgently needed to develop new research programs and new knowledge concerning the neurostructural; neurochemical; neuroendocrine and electrophysiological characteristics of affectional bonding during the formative periods of brain-behavioral development. New research

opportunities have been opened up by the discovery of the *endorphins and their known relationship to the effects of stress and the adrenocorticotrophic hormone (ACTH) where the relationship between stress and ACTH is being systematically studied by Dr. Seymour Levine, an HLB grantee. Given the special relationship between pituitary endorphin and adrenocorticotrophic hormone (ACTH) where severe stress causes depletion of pituitary endorphin and its release into the bloodstream with concomitant increased adrenocortical activity, there now appears to be new research possibilities in NICHD grant-supported research for clarifying the stress-ACTH relationship.

Although major research efforts have been directed toward understanding the role of the adrenocortical system in stress very little attention has been given to the role of the endorphin system in the mediation/regulation of stress. This relationship assumes special significance given the effect of maternal and social-isolation stress upon "emotionality", impaired affectional bonding, and the known concentrations of the enkephalins (a species of endorphins) in limbic (emotional-mediating) structures of the brain.

The research program of two HLB grantees, Dr. Seymour Levine and Dr. Joel Kaplan, are incorporating measures of endorphin activity to evaluate the relevance of endorphin activity to their studies of environmental stress, maternal deprivation, social isolation and affectional bonding. These studies may provide important new information in our understanding of the physiological mechanisms of stress, affectional bonding and their interrelationship.

*Endorphins are endogenous opioid peptides (sequences of amino acids) that are found in neural tissue and the pituitary of vertabrates. The endorphins which resemble natural and synthetic opiates have the ability to activate opiate receptors.

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Human Learning and Bheavior Branch
Contract and Collaborative Research

Contract Number: NO1 HD-1-2420

Contract Title: Research Materials and Assistance for Studies of Language Development in Children

Contractor: Haskins Laboratories, New Haven, Connecticut
Alvin M. Liberman, Ph.D.

Money Allocated: $88,079

The purpose of this contract is to continue to provide expert assistance and specialized facilities to research scientists at other institutions who are engaged in studies relevant to an understanding of the development of spoken language and reading in children.

In general, three kinds of assistance have been provided to researchers working on problems related to the acquisition of speech and reading.

1. Research Materials. Stimulus tapes for a wide variety of experiments continue to be the primary assistance that the Laboratories have provided. Several methods of generating recordings suitable for research on speech are available. The techniques can be grouped either by type of stimulus (natural speech, synthetic speech, or nonspeech tones and noises) or by type of recording (single track or dual track). All combinations of these tapes have been used for one research project or another.

2. Specialized Assistance. The professional and technical staff of Haskins Laboratories makes its knowledge and skill available to the user groups. At the very least this involves having someone from the technical staff teach the user group how to operate the computer. But it often involves, beyond that, getting various kinds of help and advice about the more scientific aspects of the research from members of the Laboratories' professional staff. In the end, the user group generates its own tape recordings.

3. Education about Speech Research. A rather high percentage of the user groups are fairly new to the field of speech. Consequently, as a by-product of their visits to the Laboratories, they have an opportunity to become better acquainted with the entire program of research underway there. Many have taken advantage of that opportunity.

Eight senior scientists reviewed the request for renewal of this contract. Without exception these reviewers recommended approval for an additional two years. There was general agreement that the service and assistance provided by this contract has been helpful to a large number of investigators many of whom would not have been able to otherwise conduct their research. There was also general agreement that there will remain for at least two years a strong need for the type of research assistance provided through this support mechanism.

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Human Learning and Behavior Branch
Contract and Collaborative Research

Contract Number: NO1 HD-7-2836

Contract Title: Prototype Test of Decoding Skills

Contractor: Bolt Beranek and Newman, Inc., Cambridge, Mass.
Drs. Ann M. Rollins and Raymond S. Nicherson

Money Allocated: $143,756

The purpose of this contract is to develop a battery of tests that can be used to determine the degree of a child's competence in the various decoding skills essential to proficient reading. After 15 months of NICHD support the contractor has completed Phase I of this project, that is, the selection of items to be included in the test batttery. During the second phase the contractor will conduct an in-depth analysis of the collected data and then revise the tests according to this analysis.

The reviewers of this contract were in general agreement that the contractor has developed a good set of six tests or tasks for measuring the major components of decoding which is reasonably comprehensive and has face validity. These 6 tasks are as follows: a. Work identification Task to Test Spelling-Sound Correspondence. b. A Use-of-Context Task--This task will be modified into a reading test to assessthe effect of context. c. The "Jabberwocky" Task--Sentences with which the content words are replaced by synthetic words are being prepared. d. Approximation-to-English Task--This task may be used as a measure of automaticity. e. Work Level Proofreading Task, and f. Reading, Fluency, or Phrasal Synthesis Task.

The reviewers also agreed that the contractors have considerable sophistication regarding the linguistic and cognitive aspects of this problem; the contractor's test battery was judged to be quite innovative and analytic with regard to the underlying decoding skills in reading.

In the second year the contractor will complete the development of the tests and then validate the battery through field testing with good and poor readers who are in grades 2 through 5 and who have I.Q.'s between 85 and 125.

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Human Learning and Behavior Branch
Contract and Collaborative Research

Contract Number: NO1 HD-7-2837

Contract Title: Prototype Test of Decoding Skills

Contractor: Research Foundation for Mental Hygiene, Inc., Albany, N.Y.
Dr. Ellis Richardson

Money Allocated: $161,324

The purpose of this contract is the development of a battery of tests that can be used to determine a child's competence in various decoding skills essential to proficient reading. During the first year of support the contractor developed a decoding test which includes three subtests: Basal Word Recognition, Phonic Decoding and Oral Readings of Passages. These three measures of decoding were administered to 120 children who were enrolled in grades 1 through 6. The data from this testing was partially analyzed during the first part of this reporting period.

Five scientists currently conducting research on the decoding process in reading and a statistician familiar with test construction procedures reviewed the progress of this contract supported research.

These reviewers were in complete agreement that adequate progess had been achieved and that the contract should be extended for another year. They felt the contractor's procedures to be used for item analysis and tests of reliability and validity were well chosen. They agreed that the contractor's staff was capable of handling the complex tasks involved in building a battery of decoding tests of reading which could be useful to researchers.

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Human Learning and Behavior Branch
Contract and Collaborative Research

Contract Number: NO1-HD-8-2824

Contract Title: Neuropsychological Studies of Reading Disabilities

Contractor: David Galin, M.D. and Charles D. Yingling, Ph.D.,
University of California San Francisco, San Francisco, CA

Money Allocated: $323,654

This contract research program is one of several activities in a program of research on reading disabilities and developmental dyslexia. The immediate objective of this contract research program is to evaluate information processing characteristics in a group of reading disabled children and matched controls utilizing quantitative methods of brain electrophysiology; neuropsychological tests and psychometric tests. Specific attention is being given to interhemispheric brain functions in the processing of simple and complex stimuli in the visual, auditory and tactile sensory modalities. A special effort is directed toward the evaluation of vestibular-cerebellar mechanism as they may relate to disorders of information processing as reflected in reading disabilities and learning disorders. The ultimate objective of this research program is to develop a parsimonious neuropsychological test protocol for the early detection and identification of potential reading disabilities in the pre-school and/or pre-verbal child. This contract research program was initiated on September 29, 1978, and has been operational for 10 months. It has been reviewed for a second year continuation.

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Mental Retardation and Developmental Disabilities Branch
Center for Research for Mothers and Children

Mental retardation ranks among the nation's most pressing social and health problems. Persisting from infancy through maturity, it ranks foremost among conditions of chronic disability. Well in excess of 200 causes of mental retardation have been identified and additional causes are being discovered regularly through research investigations. The costs of mental retardation to the Nation have been estimated at between $6.5 and $9.0 billion annually in care, treatment, and lost productivity. The greatest hope for mastery of the problem lies in its prevention and, when this is not possible, in the promotion of the individual's maximum skills through a wide-range of ameliorative, habilitative and restorative procedures.

The NICHD, within the NIH, has primary responsibility for research and research training concerned with mental retardation. This interest is expressed categorically through the Institute's Mental Retardation and Developmental Disabilities Branch (MRDD) of its Center for Research for Mothers and Children (CRMC). The MRDD Branch supports research into the biological, behavioral and social processes which contribute to, or influence the development of, retarding disorders. Of primary concern are studies concerned with finding causes and means for preventing mental retardation. Support for research pertaining to prevention of mental retardation also derives from activities of other branches of the CRMC. The Institute employs research grant mechanisms, supports special research facilities as the Mental Retardation Research Centers (MRRCs), disseminates scientific and public information, and provides contract support for research to accomplish its goals. The Institute's research programs and resources provide research knowledge and understanding applicable not only to mental retardation but to other closely related developmental disabilities as well.

Research into the causes, means of prevention, and methods for the amelioration of mental retardation serve many of the research requirements of developmental disabilities including autism, epilepsy, cerebral palsy and special learning disabilities. Children with these conditions often present with symptoms of mental retardation and, with the exception of special learning disabilities, mental retardation is frequently associated with the other disabilities.

These developmental disabilities are a frequent consequence of reproductive casualty resulting from genetic defects, disorders of pregnancy, complications of birth, and maternal ill health. The Institute, because of its assigned responsibility for research in maternal and child health, has a major research concern for these disorders. All of the Institute's programs, both Extramural and Intramural, make significant contributions relating to these issues.

Mental Retardation Research Centers

The Mental Retardation Research Centers (MRRC's) - 12 in number - were constructed under P.L. 88-164 with the mandate to conduct "research and research training in Mental Retardation and related aspects of human development". Under the provisions of the construction grant award, the centers contracted to use facilities for a minimum of 20 years for these purposes. In average, the centers have now completed about 10 years of this obligation.

The legislative language creating the centers emphasized the need for collaborative, multidisciplinary research to address the complex issues in mental retardation. Early applications for construction grants attempted to satisfy these criteria and most of the centers involve investigators from the major departments and graduate schools of the university. All but one involve the medical school in a prominent role. However, it became apparent that this model was inapplicable to some settings because of marked differences between departments in their level of interest in the problem and scientific talent. Consequently, several more narrowly focused centers were established with potential for indepth studies in some facet of biomedical or behavioral research.

As might be expected, centers are necessarily involved in common areas of investigation. While some overlap is unavoidable, and probably desirable to some degree, various approaches are employed to minimize duplication. The communication network that has been established between centers has proven to be an effective mechanism for minimizing excessive overlap of effort and promoting a cohesive national research effort.

RESEARCH HIGHLIGHTS

From its inception, the MRDD Branch has placed emphasis on developing a strong program of genetics research. Added impetus to this development occurred in FY 1978 when both the House and Senate Appropriations Committees included language in their reports urging the Institute to increase its research in genetics, specifically stipulating research in Down syndrome. Data supportive of continued emphasis on genetics/Down syndrome research are compelling and advances realized and expected from Branch contract and grant supported research encouraging.

An Interregional Cytogenetic Registry was established with contract funds at the University of Oregon Health Sciences Center in Portland. Established with the objective of designing and maintaining a registry of clinical and cytogenetic information, the registry will be instrumental in providing better health care delivery. These goals can be achieved directly through registry use in the delivery of genetic services as diagnosis, counseling, and follow-up, and indirectly as an aid to genetic research.

To these ends, registry data can be utilized to provide information on morbidity, etiology, recurrence risk estimates, frequency of break points, genetic linkage and gene mapping, karyotype/phenotype correlation, mutation rate estimates, genetic counseling, health care needs and delivery.

With the collaboration of five additional and widely dispersed cytogenetics laboratories, the registry design has been successfully accomplished. Standards of uniform recording and reporting of data have been established which have been shown to be valid and reliable across collaborating laboratories. A manual has been developed which is finding broad acceptance by laboratories across the country and abroad.

A major deterrent to a fuller application of cytogenetic knowledge has been the time and effort required to perform chromosome analysis in the laboratory. This challenge has been met through the development of automated procedures for chromosome preparation and karyotyping. This work has been accomplished through use of three separate but related contracts.

A semi-automated system for chromosome preparation has been successfully developed by the contractor at the City of Hope Medical Center, Duarte, California. This system offers major advantages over other systems including (1) ability to process large numbers of specimens; (2) uniform treatment of all specimens; (3) ability to process controls and replicates in same tray; (4) strict control over specimen identification; and (5) reduction in cost and time of processing.

A stand-alone automatic karyotyping device has been developed under contract by the Jet Propulsion Laboratory in Pasadena. Operation of the automated system provides for four major activities including (1) slide search (that is, selection of metaphase spreads); (2) scanning; (3) spread analysis; and (4) generation of a karyotype. This device, when linked operationally with the semiautomated system for chromosome preparation, provides complete automated system capable of meeting the burgeoning needs for chromosome analysis in clinical genetics and cytogenetic research.

The complete system is now undergoing field testing and updating under a contract let to Northwestern University. This testing and upgrading of the complete system will enchance its capability by incorporating recent technical and scientific developments in cytogenetics and image processing thus making the system more versatile and responsive to the cytogenetic community it is intended to serve.

With an incidence of about 1 per 700 live births, Down syndrome is the most common and readily identifiable genetic condition characterized by mental retardation. Despite its recognition as a distinct clinical entity, and the first condition to be associated with chromosomal defect, several fundamental issues concerning this issue continue to elude scientific explanation.

Research restraints on use of human subjects have prompted the Branch to turn to the development of animal models holding promise for providing understanding of the mechanisms involved in Down syndrome. Here, research advances have demonstrated capability for systematic study of trisomy using animal models.

Studies in Infants at Biological Risk

Among infants at risk, premature infants are a puzzling group. Not only do they frequently have health crises at birth but they also are at high risk for later developmental disabilities. How these two facts relate is still unknown, however. The state of a premature baby's health frequently does not correlate with the extent of later disabilities, and there is often a long gap between birth and the time handicapping conditions appear. This greatly complicates matters for health professionals caring for such infants.

Investigators at the Neonatal Intensive Care Unit in Washington have been attempting to shed light on this and related problems in a five year longitudinal study in order to improve predictive abilities and to diagnose/intervene at a much earlier age than has previously been possible. Infant subjects include: (1) Survivors of hyaline membrane disease; (2) Those with birth weights of 1500 grams or less; (3) Those with central nervous system (CNS) infection such as meningitis.

Results so far show that of the 250 children who have been seen in the NICU, 20-25 percent have a recognizable developmental handicap by the age of three, with cerebral palsy the most prevalent. Investigators usually find signs of CP at the four-month visit and, in the great majority of cases, are able to make a definitive diagnosis at the eight-month visit. The mean age at which CP is diagnosed in the community at large is 18 months, and children are generally two years of age before they start receiving treatment. It is especially interesting to note that most of these cerebral palsy victims were thought to be normal when they left the hospital after birth.

The second most prevalent disability found by the investigators is developmental delays/mental retardation with 15-20 children so diagnosed. Most of these children are now enrolled in early infant stimulation and language stimulation programs.

At Albert Einstein College of Medicine in New York, analysis of prospective data in a population of 150 low-birth-weight infants has clearly demonstrated that the head circumference at birth is the best predictor of later intellectual function and is a much better forecaster than birth weight. Fetal growth retardation which spares head growth is associated with significantly better neurologic outcomes than is fetal growth retardation which also reduces intrauterine head growth. While this is not at all surprising, what is surprising is that this has never been previously demonstrated.

Neurosciences

Research in the neurosciences is clearly central to an understanding of mental retardation and is a major focus of MRDD Branch activity.

In order to define fine structural changes in cerebral cortex which may form the anatomical basis for mentally retarded individuals in whom no gross developmental or destructive lesions are evident, an investigator from the University of Chicago has been studying synaptic and dendritic development in cerebral cortex. Using phosphotungstic acid method in tissues fixed up to 36 hours postmortem, synaptic density was shown to be constant throughout adult life (ages 16-72 years). There was a slight decline in synaptic density in the brains of the aged (74-90 years). Synaptic density in neonatal brains was already high, in the range seen in adults. However, synaptic morphology differed; immature profiles had an irregular presynaptic dense band instead of the separate presynaptic projections seen in mature synapses. Synaptic density increased during infancy, reaching a maximum at age 1-2 years which was about 50% above the adult mean. Synaptic density declined between ages 2-16 years, accompanied by a slight decrease in neuronal density. Although the full significance of overproduction of synapses during infancy is not fully understood, this phenomenon could, perhaps, explain neuronal "plasticity" in the developing child.

An investigator at the University of Wisconsin, in studying the structure of the hypothalamus, has discovered a group of cells which appear to act as scavengers, protecting the brain from virus infections. As is true for other organs, however, there may be a critical period during which these scavenger cells are not fully developed or functioning. Identification of this critical period--under investigation--has definite implications for the prevention of brain damage caused by a virus infection in the mother or infant.

Teratogenic and Toxic Agents

Several investigators are devoting considerable effort toward assessment of the potential of drugs and chemicals to cause congenital defects. Teratological testing has traditionally been carried out in laboratory rodents, but it is well recognized that species and individuals often exhibit dramatic differences in their teratogenic responses to particular compounds. Numerous studies of the serious human teratogen thalidomide, for example, have shown that most test animals exhibit greatly reduced or absent sensitivity to its embryotoxicity. A first priority in teratologic research, therefore, must center around the development and testing of new systems with enhanced predictive validity for human exposure.

Investigators at the University of Washington are studying environmental pollutants and drugs to which pregnant women are exposed which are comprised of organic compounds likely to be metabolized in the maternal liver. All embryos and most fetuses of the species which have been examined show virtually no ability to metabolize these foreign substances, relying

instead upon maternal mechanisms. The primary function of this hepatic metabolism is the elimination of foreign compound from the body. Although hepatic metabolism may result in the inactivation of compounds, it may also lead to the generation of toxic metabolites. These investigators have postulated that the form and severity of congenital defects resulting from drug and chemical exposure are related to the toxicity of the specific metabolites reaching embryos, rather than to the embryonic metabolism per se. Further tests using human liver are planned to assess the utility of a nearly developed system as a human screening system.

Although the potential teratogenic effects of alcohol have been suspected for centuries, it was not until the work of Lemoine in 1967 (France) and the observations of Jones and Smith in 1973 (Washington) that a distinct, dysmorphic condition associated with maternal, gestational alcoholism was described. Extensive animal studies have now demonstrated the specific teratogenic properties of ethyl alcohol in a variety of species, many of the abnormalities being similar to those found in man. Because of the widespread use of alcohol, the potential magnitude of birth defects and developmental retardation stemming from ethanol exposure has become an object of inquiry. Although the data are still too fragmentary for reliable estimates, recent observations suggest an incidence of fetal alcohol syndrome between 1 and 2 per 1,000 live births, with partial expression of the disorder in perhaps 3 to 5 per 1,000 births. Experience at the Genetics Clinic in this center during the past few years supports the impression of a more widespread prevalence of the problem than has been appreciated.

The abnormalities associated with the fetal alcohol syndrome involve a variety of organ systems, and are manifest as growth deficiency, a characteristic cluster of facial stigmata, and central nervous system dysfunction. The latter, characterized mainly by mental retardation, is one of the most commmon and serious problems associated with ethanol teratogenicity. Although not all persons with the fetal alcohol syndrome are retarded, rarely has an affected patient been described with average or better than average mental ability. Indeed, it has been claimed that maternal abuse of ethanol during pregnancy may be the most frequent known teratogenic cause of mental retardation in the Western world.

Exposure to lead is one of the most serious health problems for American children. It has been estimated that as many as 600,000 American children under 6 years of age have evidence of "undue lead absorption", i.e. blood lead levels 30-69 ug/dl. Children who have had lead poisoning and recover are frequently left with profound developmental impairments, including mental retardation, seizures, cerebral palsy, and behavior disorders. Recent research advances have drawn attention to the effects of sub-toxic lead levels on development which call for reconsideration of new standards for acceptable lead levels.

To evaluate the neuropsychologic effects of unidentified childhood exposure to lead, an investigator from the Children's Hospital Medical Center in Boston studied neurobehavioral performance in a group of children in relation to lead exposure. The children were in the first

and second grades selected from two communities in Massachusetts all of whom were considered asymptomatic for lead intoxication. Children were ranked in the highest and lowest 10th percentile for lead concentration in the dentine of shed deciduous teeth. Their neuropsychologic performance was evaluated in the investigator's laboratory. In addition, their classroom behavior was also measured by teachers' ratings.

Children with high lead levels scored significantly less well on a standard psychological test than those with low lead levels. Verbal performance and auditory processing appear most vulnerable. Teachers' reports of classroom behavior showed that children with high lead levels were rated significantly poorer on 9 of 11 items. The frequency of non-adaptive classroom behavior increased in a dose-related fashion to dentine lead level. This study suggests that lead exposure, at doses below those producing symptoms severe enough to be diagnosed clinically, appears to be associated with neuropsychologic deficits that may interfere with classroom performance.

Infant Development

Research conducted in the infant laboratory, University of Wisconsin has revealed that the infant listener is impressively well-equipped and developed in the first few months of life to detect, discriminate and even categorize many of the basic units of human speech and sounds which soon thereafter begin to emerge in the infant's babbling and words. Using heart rate response and a non-nutritive sucking procedure, even prematures and other infants experiencing complicated births were able to make meaningful discriminations.

A behavioral researcher in the Child Development and Mental Retardation Center (Washington) has been making important discoveries about the perceptual and cognitive sophistication of newborn babies. These discoveries provide answers to old theoretical puzzles, and open new avenues for infant assessment procedures.

This scientist's most recent work concerns the infant's capacity for "cross-modal matching". Normal adults judge two identical objects to have the same shape when they touch one and see the other. The origin of man's ability for such "cross-modal matching" has been disputed for centuries. The prevalent view is that children gradually learn to associate visual and tactual sense impressions through repeatedly reaching out and touching objects they see. Experiments now show that 28-day old babies can already solve cross-modal matching problems, thus suggesting that this is an innate rather than learned ability.

Most importantly, studies indicated that neonates' perceptual-cognitive systems are not limited to processing modality, specific information such as retinal images or tactual sensations. Rather, the neonate seems to be capable of storing abstract representations of the world--representations that are abstract enough, at least, to transcend and unify sense impressions originating in different modalities.

Important clinical applications based on these findings are quite probable. Research with children and adults has already documented deficiencies in cross-modal functioning in retarded readers, brain-damaged persons, schizophrenic children, and psychiatrically disturbed adolescents. The present research provides a test of cross-modal functioning that can be used with infants in the first month of life. Such a tool may provide a means of identifying potential mental and sensory defects at an age much earlier than previously thought possible.

Prenatal Diagnosis

The importance of prenatal diagnosis as a preventive measure in mental retardation is well established, but the invasive nature of the procedure may pose barriers to its widespread application by physicians and full utilization by women for whom the procedure may be indicated.

Alternatives or adjuncts to amniocentesis have been investigated by investigators at Albert Einstein College of Medicine on a consecutive series of 800 women referred for this procedure. The results were compared before and after introduction of sonography immediately prior to the tap as routine adjunct of the procedure. Following use of sonography there was a statistically significant reduction in the frequency of bloody taps, and an increase in the proportion of successful and informative taps. Moreover, in many instances sonography provided information useful for identification of fetal abnormalities or for management of pregnancy. These findings support the recommendation that examination by sonography should be utilized as an adjunct to amniocentesis.

The widespread use of midtrimester amniocentesis for diagnosis of fetal cytogenetic or biochemical abnormalities has inherent limitations because of the effort, expense, and frequent delay involved in growing amniotic cell cultures required for analysis. Moreover, the amniocentesis procedure involves a small risk (generally assumed to be less than 0.5%) to the fetus and less so to the mother. A procedure involving isolation and study of fetal lymphocytes from the maternal circulation early in mid-trimester may provide a more useful and widely applicable method to screen for fetal genetic abnormalities.

During earlier studies, Shriver Center (Waltham) scientists evaluated the application and assay for alpha-fetoprotein in amniotic fluid, moving it from the research bench to a routine test. They have now examined over 14,000 cases, diagnosed 110 neural tube defects and missed no open lesions. Parallel studies with maternal serum alpha-fetoprotein have now been completed on over 6,000 women. They show a detection efficiency of 87.5% for detecting neural tube defects. Twins between 16 and 18 weeks gestation were detected in almost 50% of such cases. The data also show that maternal serum alpha-fetoprotein studies may prove to be an important tool in the management of pregnancy and in the detection of women at high, but unsuspected, risk.

Intervention Studies

Young, black women, seriously disadvantaged in childhood, who participated in an early education program more than a dozen years ago, seem to have retained positive attitudes toward school achievement even after becoming adolescent mothers.

Followup study in 1978 showed that women in the experimental group were significantly more likely to finish high school and complete their education following pregnancy than control women. The researchers point out that if motivation to complete school in spite of childbearing is related to the very early educational experience, then the effects "are greater and more lasting than the common wisdom has assumed".

A more intensive intervention program with black children in rural, extremely impoverished areas of North Carolina is now entering a followup, school-age period. These children, who were enrolled in early infancy in an educationally oriented day care setting, score significantly higher on intelligence, language, and related tests than their controls. Using a cross-over design, half of the experimental children will receive family-based, continuing enrichment experiences while in public school to determine whether those gains can be sustained over time.

Replication in North America of an Israeli educational program (previously supported by NICHD) designed to help slow-learning adolescents change their thinking processes has resulted in IQ gains after only one-fourth of the teaching program has been completed. The results of Instrumental Enrichment, as the program is called, are being studied at the Kennedy Center for Research on Education and Human Development (Nashville). An average of four hours a week was provided of systematic classroom instruction in fundamental thought processes, logic, and problem-solving strategies to 14 classes of adolescents in special education programs in Nashville and Louisville. Similar classes were conducted by colleagues in Toronto.

After only five months and an average of 50 hours of instruction in the two year Instrumental Enrichment program adolescents classified as educably mentally retarded, learning disabled or behavior-disordered rose in mean IQ from 7-9 points. On the letter series subtest of Thurstone's Primary Mental Abilities Test, students in the experimental program improved their performance by 62.8 percent; performance of students in the control group declined slightly.

The results are similar to those obtained in Israel with larger groups in the 250 to 300 hours required for the whole program. The investigators anticipate further IQ gains, as well as gains in academic achievement and motivation to learn, as the program proceeds. They observe that the data collected in North America and in Israel show that adolescence "is not too late" to change the cognitive patterns of slow learners.

Dietary Treatment

UCLA scientists have found a substantial and positive clinical response can be obtained from dietary therapy in hyperargininemia, a progressively debilitating neurological disorder, even with the institution of therapy at a late age and at a late stage of the disease. This fact suggests that if instituted earlier, hyperargininemia due to arginase deficiency may be successfully treated and the substantial majority of symptoms mitigated. These findings are quite hopeful for the patients, but present a considerable dilemma to the physician who may now have to exert a great amount of effort to treat non-compliant patients.

From the University of Cincinnati, promising new treatment for phenylketonuria (PKU) has been described. Administration of branch chain amino acids (valine, isoleucine, leucine: VIL) alone or in combination with the low phenylalanine diet, consistently reduced the CSF/serum phenylalanine ratio in phenylketonuric patients. This work confirmed previous animal studies in which VIL treatment reduced the entry of phenylalanine into brain of rats with experimental PKU. Application of the new treatment may prevent the intellectual deficit now seen even with early treatment of PKU and permit the diet to be liberalized for older PKU children.

A collaborative study of children treated for PKU is being conducted in 15 medical centers across the United States. The study includes 140 PKU children who were treated with a low phenylalanine diet shortly after birth to six years of age. Comparison of 51 PKU children with their nonaffected siblings matched in age (72.2 months) at the time of testing showed a mean Stanford Binet IQ of 97 for the PKU children and 103 for their siblings.

At age six, with the consent of the parents, each child was randomly assigned to one of two groups, either to continue on a phenylalanine-restricted diet, or to discontinue and be free to eat a normal diet. The effects of diet continuation/ discontinuation were evaluated for 37 and 46 PKU children at age seven, respectively, and for 27 and 28 PKU children at age eight, respectively. Results of the analysis failed to show any evidence that continuing the diet was detrimental. In fact, in one analysis, the mean IQ of the continuation group increased from 97 at age six to 101 at age eight. The PKU children in the discontinuation group had lost one IQ point, from a mean of 97 to 96 by the end of the first year, and five IQ points, from 96 to 91, by the end of the second year off the diet. The difference between the groups at seven years was not statistically significant, but at eight years it was significant. It was the opinion of two independent groups of reviewers that a low phenylalanine diet is helpful up to the age of 6 years, and it is safe to continue the diet beyond age six. It was also felt that there are insufficient data that would indicate that diet discontinuation at age six is harmful. Continued and careful follow-up is necessary, particularly the school performance, socialization and emotional development of the children, not just the effect of the diet on IQ.

A summary of the Branch portfolio is shown in the following table:

Mechanism of Support	Number of Projects	Funds (thousands)
Research Grants (R01)	84	$ 6,878
Program Projects (P01)	29	11,687
Center Grants (P30)	12	6,729
Research Career Program Awards	4	145
Scientific Evaluation Contracts (R09)	5	1,033
Total Research	135	26,553
Training Grants (T01)	9	806
Fellowships:		
F32	6	91
T32	8	1,003
Total Training	23	1,900
Total Research and Training	158	28,453

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Mental Retardation and Developmental Disabilities Branch
Center for Research for Mothers and Children
Collaborative Research and Contracts
Summary of Reports

Contract Number: NO1 HD-7-2847

Contract Title : The Impact of Foster Family Care in the Development and Behavior of Mentally Retarded Children

Contractor : Regents of the University of California at Los Angeles

Money Allocated: FY '79 - $ 103,303.00

The purposes of this contract are to determine:

1. Characteristics of children most likely to profit from foster family care.

2. The kind of socialization and developmental experiences afforded to such children.

3. The qualifications and characteristics of foster parents conducive to successful placement.

4. The most appropriate and effective roles of foster family members, natural parents and agency workers.

5. Special considerations and community supports needed to adapt foster family care to needs of the retarded.

6. Utilization of foster family care for long term placement.

This project is about to enter the final year of proposed work. Data collection will continue for another six months with the remaining time devoted to data analysis and reporting. The work is on schedule and is being conducted in an exemplary manner, including the presentation and/or publication of preliminary findings in ten separate reports.

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Collaborative Research and Contracts
Summary Reports

Contract Number: NO1-HD-32713

Contract Title: Coordination of Regional Cytogenetic Registries

Contractor: University of Oregon Health Sciences Center

Money Allocated: FY 1979 - $242,853

This contract was initiated to develop a common format for the collection of clinical and cytogenetic information; to establish defined standards in the selection of material for analysis and for quality control; to develop a manual of procedures which will contain information about standardization in the selection of material for analysis and for maintenance of quality control; and to develop a phenotype coding system. The registry is designed to maintain a record of genetic and clinical information which will help provide better health care services, and to provide a data base for research and epidemiologic studies.

Records on about 25,000 patients have now been entered in the registry. Over 8,400 of these patients have cytogenetic aberrations and about 2,000 have heteromorphisms.

Projects have now been initiated using data retrieved from the registry, and will address the following issues:

1. To determine the temporal patterns in the relative mutation rate of interchange trisomy 21 and 13, and if the regional patterns are comparable.

2. To characterize the rates of chromosomal conditions diagnosed prenatally by single year maternal age interval, so as to provide baseline data for surveillance and monitoring for these conditions; to provide data pertinent to genetic counseling; and to estimate the differential rates found at amniocentesis and cases diagnosed in live births.

3. To determine the frequency, type and significance of "spurious cells" in amniotic cell culture.

4. To determine the frequency of mental retardation in Turner syndrome, and to compare the maternal and paternal ages in cases of Turner syndrome.

5. To determine the clinical distinctions, if any, between classic (XO) Turner syndrome, and patients with structural abnormalities of the X chromosome, including XO/autosome translocations.

6. Phenotype-karyotype correlations.

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Center for Research for Mothers and Children
Collaborative Research and Contracts
Summary Reports

Contract Number : NO1-HD-8-2858

Contract Title : Development of an Animal Model for Genetic Disorders with Emphasis on Trisomy 21 (Down Syndrome)

Contractor : University of California, San Francisco

Money Allocated: FY 1979 - $183,187

The objectives of this contract, which was initiated on September 30, 1978, were: 1) to expand existing mouse colonies in order to enhance trisomy production; 2) initiate isolation of new metacentric chromosomes; 3) analyze the frequency of production of the various trisomies; 4) freeze pre-implantation embryos; 5) map human chromosome 21 homologous loci in the mouse genome; and 6) analyze the effects of chromosomal aneuploidy on the development of the brain and other organs and tissues.

The mouse colony in San Francisco presently includes eight homozygous and six double heterozygote translocation stocks. In Lubeck, the subcontractor to this project, 26 translocation stocks isolated from feral mice are available, with an additional four stocks of laboratory origin. About 80 wild mice have been scored and typed for metacentrics, 22 of which have been added to the colonies for future isolation of metacentrics. The investigators have identified the following factors which influence segregation patterns: female heterozygotes have a higher frequency of abnormal anaphase one segregation than do males; the genetic background, i.e. the specific chromosomes involved, seems to affect segregation, and the time of lethality. Excellent progress has been achieved in mapping human chromosome 21 loci homologous to the mouse genome. Superoxide dismutase (SOD-s) assigned to human chromosome 21 has been assigned by other investigators to mouse chromosome 16.

Experiments carried out under this contract has demonstrated synteny between mouse interferon receptor and SODs which, if confirmed, would prove that two out of the three human chromosome 21 marker loci (the third being glycinamide ribonucleotide synthetase) would have been assigned to mouse chromosome 16. This would suggest that the genetic material of mouse chromosome 16 bears some homologous relationship to human chromosome 21. Morphological and histological analyses of the effects of chromosome aneuploidy on development of the brain and other organs are being pursued in Lubeck.

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Mental Retardation and Developmental Disabilities Branch
Center for Research for Mothers and Children
Collaborative Research and Contracts
Summary Reports

Contract Number	:	NO1-HD-8-2855
Contract Title	:	Field Testing for Chromosome Preparation and Karyotyping Device
Contractor	:	Northwestern University
Money Allocated	:	FY 1979 - $252,500

The objectives of the contract, which was initiated on September 30, 1978, are to carry out a comprehensive evaluation and testing of an automated karyotyping device which was developed at the Jet Propulsion Laboratory, and a semi-automated system for chromosome preparation which was developed at the City of Hope Medical Center. The project consists of three phases. The first phase consists of evaluating, in a series of "blind", controlled trials, the two systems. After the initial evaluation, modifications will be made prior to clinical and research testing. Software development for karyotyping and for the detection of chromosomal aberrations will be carried out. Phase two will be devoted to an extensive clinical and research testing of the automated systems. Blood specimens and amniotic fluid from various sources will be cultured and karyotyped using the automated devices. Modifications of the hardware, incorporating current technology, will be completed during phase three. Further testing of the systems will continue during this phase.

Although the investigators have found many deficiences in the two systems, they can all be overcome by either new equipment, which is now becoming available, or by modification of the current hardware and software. After initial delays in transfering and installing the equipment to its present site, the investigators have now begun to evaluate, improve, and test the devices.

Center for Research for Mothers and Children

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Office of the Director
Epidemiology and Biometry Research Program

The Epidemiology and Biometry Research Program (EBRP) conducts research on the epidemiology of disorders of mothers and children, of perinatal mortality and morbidity and of abnormalities of human growth and development. It also provides consultation to other programs of the Institute.

A program director has just been appointed. The program is small and understaffed and vacant positions for which additional professional staff was being recruited are caught in the employment freeze. The record of performance for the past year is presented in the following report of the three program components.

Computer Unit

The Unit on Systems Design and Data Processing is charged with the responsibility of developing and implementing systems for handling the many studies in the Epidemiology and Biometry Research Program area. Such responsibilities and involvement include the major commitment of system staff, time and other data processing resources, from the exploratory stages of an investigation to the final analysis and study reporting.

The section has provided support to a clinical trial that makes use of phototherapy in the treatment of neonatal hyperbilirubinemia. A data base of over 18,000 records to date have been created. All records before entering the file passes through a system, editing the records for error, gross analysis of the records for inconsistency and many tabulations tracking the effects of phototherapy as a treatment.

A study of breast feeding in relation to the child's health was performed utilizing data obtained from a Pima Indian Population. The study was conducted in two phases. A pilot phase, consisting of twenty-five mothers and the full investigation, consisting of three hundred fifty-seven mothers. A data-base consisting of records on the socio-economic status of the mother, her pregnancy history, medical history, and data for her current pregnancy was created. Tabulations of breast, bottle and breast and bottle feeding with other demographic variables were made.

The section provided support for a study of high infant mortality in a nearby metropolitan area designed to determine the relationship of environmental factors to the cause of death for infants who died in the first year of life. To insure completeness of data, a system was established to measure data during all stages from data collection to completion of the project. The system also reviewed continuously the procedure for handling of the data. The data base created contains data of the live births, infant deaths and fetal deaths for the metropolitan area for the years 68 through 73. The data base contains information on 200 environmental variables specifically for the metropolitan area for the stated years.

A randomized clinical trial designed to assess the therapeutic value of ascorbic acid in the treatment of early onset cystinosis was terminated early in November, 1977. However, data follow-up on participants are continuing. The data base from which a final report has been written will continuously be maintained by the section. Tabulations will be made to measure data set accuracy.

Studies using information from linked birth and death certificates for years 1967 through 1973 for several states in the U. S. were processed by the Section on Systems Design and Data Processing. The records include all of the live births, infant deaths and fetal deaths along with other demographic variables for the stated years. Sophisticated editing, updating procedures have been written. In addition analysis of mortality as a function of maternal age showing the risk of birth weight specific mortality in young mothers, have been performed.

Acknowledgements:

1. Schneider, J. A., Schlesselman, J. J., Mendoza, S. A., Orloff, S., Thoene, J. G., Kroll, W. A., Godfrey, A. D. and Schulman, J. D.: Ineffectiveness of ascorbic acid therapy in nephropathic cystinosis. New Engl. J. Med. 300:756-759, 1979. With acknowledgements to: Harley, Ernest E., Roberts, Inez M. and Nelson, Elva.

ANNUAL REPORT
October 1, 1978 through September 30, 1979
Epidemiology Branch
EBRP/NICHD

During the past year there have been several staff changes in the Branch. One part-time research assistant, Michael Reid, served as a member of the professional staff, and Dr. Susan Harlap began a four-month period as Visiting Scientist from Israel. A temporary summer student has been hired to participate in field work in Arizona from July through September, 1979, and Dr. Jim Mills has been assigned to this office as a Clinical Scholar. Full-time professional staff currently consist of Drs. Keller and Forman, and Robert Nugent.

Although no new research projects are currently involved, interest in response to national concern over the effects of chemical toxins on reproductive efficiency has continued. Other areas in which contract proposals are being developed include teen-age and other high risk pregnancy, and breast feeding in Israel and North Africa in transitional populations. Projects in which members of the Branch have or have had primary responsibility during the past year include:

1. The Epidemiology of Infant Mortality in Baltimore, MD.
2. The Relationship of Body Density to Other Methods of Assessing Obesity in Eight Year-Old Children and the Association of Obesity with Present and Past Nutritional and Activity Factors and Parental Attitudes.
3. Breast and Bottle Feeding in the United States: The 1978-79 National Natality Survey.
4. Breast and Bottle Feeding in the United States: The 1969 National Natality Survey.
5. The Geographic Variation of Fetal and Infant Mortality and Low Birth Weight in the United States, 1969-1974.
6. The Effect of Illegitimacy on Infant Mortality from Linked Birth-Death Records in Minnesota, 1967-1973.
7. Digital Preference of Conceptions in Missouri, 1968-1973.
8. The Seasonality of Infant Mortality From Linked Birth-Death Records in North Carolina, 1967-1973.
9. Infant Feeding Among the Pima Indians: Time-trend, Factors Associated with Infant Feeding and Its Effect on Child Health.

A brief description of each of these projects is included in the next section. Additional activities of professional staff of the Branch included chairing a session at a national meeting, presenting four papers at national meetings, consulting with NIEHS, and other routine intramural and ad hoc committee assignments. Invited presentations have been made at the Argonne National Laboratory, The University of Maryland School of Medicine, The Brooklyn (NY) Lutheran Medical Center, and an NIMH-sponsored workshop on prematurity. In addition, one member of the professional staff is currently serving on a National Research Council Committee which will meet on at least two occasions in FY 1979.

PROJECT NUMBER (Do NOT use this space)	HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	CONTRACT NUMBER: N01-HD-7-2845

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)
The Epidemiology of Infant Mortality in Baltimore, MD

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Project Officer:	Carl A. Keller	Acting Chief	EB/EBRP/NICHD
P.I. :	Genevieve Matanoski	Professor	Johns Hopkins Univ.
Other :	Michele R. Forman	Sr. Epidemiologist	EB/EBRP/NICHD
	Robert P. Nugent	Research Assistant	EB/EBRP/NICHD

COOPERATING UNITS (if any)
The Johns Hopkins University School of Public Health

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.45	.45	

CHECK APPROPRIATE BOX(ES)

XX (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS XX (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This contract was designed to study the simultaneous effects of a number of risk factors for infant mortality in a total community. The contractor was let pursuant to an RFP which specified that the study be conducted in a high risk community within 50 miles of NIH so that Branch staff could collaborate with the contractor frequently on a regular basis. The results of this study will be used to assess the relative importance of a number of risk factors for infant mortality which might be incorporated in the design of an intervention program.

PHS-6040
(Rev. 10-76)

1. Contract Description:

This contract was awarded to Johns Hopkins University School of Public Health on September 30, 1977 for the 2-year period through September 29, 1979 in the total amount of $333,250.00.

The contract is a three-part process and a description and progress are as follows:

i) A case-control study of all infant deaths in the city of Baltimore for a one-year period starting September 1, 1978. Approximately 250 mothers of infants who have died and 500 mothers of live infants will be interviewed using a questionnaire designed by the contractor and Epidemiology Branch staff. The interview covers all of the known risk factors for neonatal deaths and many of those for post-neonatal deaths, plus a number of other issues relating to phychosocial supports, preparations for birth and accessability to medical care at the time of death of the infant.

Progress to date has included making arrangements with Baltimore hospitals for selections of cases and controls, and completion of the questionnaire by OMB. Interviewing of cases and controls began in December 1978, using deaths occuring in November.

ii) A detailed examination of the causes of death reported on the death certificate and comparison with hospital records and other information on all deaths during a two-year period beginning October 1, 1978.

The administrative aspect of this phase is currently being conducted by the contractor and progress is satisfactory to date.

iii) An environmental study using demographic and environmental variables to determine the possible relationship of these variables to fetal and infant death rates and low birthweight ratios by census tract for the metropolitan Baltimore area for a 5-year period.

Data collection for this phase has been completed and collaborative analysis is now underway with Monroe Lerner of Johns Hopkins University and Epidemiology Branch staff.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	XXXXXXXXXXXX CONTRACT NUMBER: N01-HD-7-2848

PERIOD COVERED

October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Relationship of Body Density to Other Methods of Assessing Obesity in Eight Year-Old Children and the Association of Obesity with Present and Past Nutritional and Activity Factors and Parental Attitudes.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Project Officer:	Carl A. Keller	Acting Chief	EB	NICHD
P.I. :	Ruth Huenemann	Professor	Univ.Cal.,	Berkeley
Co-P.I. :	William Bruvold	Assoc.Prof.	Univ.Cal.,	Berkeley

COOPERATING UNITS (if any)

University of California, Berkeley; School of Public Health

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.20	.20	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The measurement of obesity in children is not a universally accepted process. Circumferences, skinfolds, height, and weight have been used in adults and correlate well with more definitive measurements based on body density or fat content, but the same procedures have not been tested adequately in pre-pubescent children. This project was designed to make simultaneous measurements using all of these procedures, including underwater weighing, and to correlate these with previous measurements on the same children and with food intake histories, activity scores, and parental attitudes toward food consumption.

PHS-6040
(Rev. 10-76)

1. Contract Description:

This contract was awarded to the University of California School of Public Health, Berkeley, California on September 30, 1977 for a period of 18 months in the amount of $77,331.

The study population consists of all children remaining in a cohort of births to residents of Berkeley, California in 1969 and on whom anthropometric, food history, activity, and parental attitude data have been collected at one to two-year intervals to the present.

The objectives of this study are to develop a valid method for the measurement of body fat in eight to nine-year old (pre-puberty) children. Practical suggestions for the use of anthropometric measurement of pre-pubescent obesity which can be accomplished with available clinical instruments will be determined. In addition, these measurements will be related to previous eating and activity practices as well as previous anthropometric measurements on the same children to assess the effects of early feeding and activity and the predictive values of early measures on pre-pubescent body fat content.

The results of this study should provide data for more accurate clinical measures of childhood obesity and early predictors for these measures. It is planned to perform data analysis at NICHD.

An extension of 3 months, without additional funds, was granted in order to allow better organization of data to be submitted to NICHD for final analysis. Two of three tapes have been received with documentation and are currently being analyzed and the final tape is due soon. A final report on this subject will be completed during FY 1980.

PROJECT NUMBER (Do NOT use this space)	HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD-00302-02 EB

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)
Breast and Bottle Feeding in the United States: The 1978-79 National Natality Survey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I. :	Michele R. Forman	Senior Epidemiologist	EB/EBRP NICHD
Other:	Ernest Harley	Computer Specialist	CS/EBRP NICHD

COOPERATING UNITS (if any)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.05	.05	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Participation in the National Natality Survey of the NCHS will provide data on current breast feeding practices in all segments of the population. Comparison with data from the 1969 survey will provide for the assessment of changes in breast and bottle feeding over the last decade.

PHS-6040
(Rev. 10-76)

1. Project Description:

Three questions on the attitudes about and social influences surrounding breast feeding practices have been submitted and are included in the current National Natality Survey. This survey, currently in progress, will follow up 6,000 births at approximately 3 months of age with a mailed questionnaire to a 1 in 500 sample of mothers and will include demographic and some medical information on each birth. Data will be analyzed to assess which factors are associated with breast feeding and to estimate the current status of breast feeding in the United States.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD-00303-02 EB

PERIOD COVERED

TITLE OF PROJECT (80 characters or less)

Breast and Bottle Feeding in the United States: The 1969 National Natality Survey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I. :	Michele R. Forman	Senior Epidemiologist	EB/EBRP	NICHD
Other:	Ernest Harley	Computer Specialist	CS/EBRP	NICHD

COOPERATING UNITS (if any)

LAB/BRANCH Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.33	.33	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The analysis of data from the 1969 National Natality Survey conducted by NCHS has provided a basis for measuring trends in breast and bottle feeding practices which may have occurred over the past ten years.

PHS-6040
(Rev. 10-76)

1. Project Description:

A questionnaire was mailed to a stratified random sample of women with legitimate live births in 1969 in the United States. This sample made up the National Natality Survey and 3,611 women provided information about the type of infant feeding--breast and/or bottle. Data was examined by socio-economic status, parity, mother's age, gestational age and birth-weight of the newborn, and prenatal care, and results of the analyses were stratified by race.

Major findings include a higher proportion of breast feeders (18 vs. 9%) among the whites compared to the blacks. There was a strong positive association between higher education and income, and first births among the white breast feeders. Black women aged 30-34 years and parity 5+ who are also in a high income group tended to breast feed more than other black women, and in both groups, the premature babies were breast fed the least. These findings have been submitted for publication and will be compared to current practices to measure the changes during the past 10 years.

PROJECT NUMBER (DO NOT use this space)

HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

Z01-HD-00304-02 EB

PERIOD COVERED

October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Geographic Variation of Fetal and Infant Mortality and Low Birth Weight in the United States, 1969-1974.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I. :	Carl A. Keller	Acting Chief	EB	NICHD
Other:	Karen Fetterly	Computer Specialist	CS/EBRP	NICHD
	Michael Reed	Research Assistant	EB	NICHD
	Kathleen Thomas	Secretary	EB	NICHD

COOPERATING UNITS (if any)

LAB/BRANCH

Epidemiology

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.50	.45	.05

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The assembly of a large number of variables, including pregnancy outcome, has provided data on the marked variability in infant and fetal mortality among different populations in the U.S. The correlation of adverse pregnancy outcome rates with environmental variables will make it possible to assess the relative important of the various factors associated with these rates in a large population. These data will also provide a basis for the assessment of changes in infant and fetal mortality which may be occurring in some populations.

PHS-6040
(Rev. 10-76)

1. Project Description:

Approximately 80 variables have been assembled for each of the 3,078 counties in the United States and include data from the 1970 census and other sources pertaining to environmental and geographical characteristics of each county. The file also includes births, fetal and infant deaths, mother's age, parity, and race.

Analysis will include multivariate statistical procedures and description of various factors associated with high and low rate of adverse pregnancy outcomes and should be completed by the end of FY 1980. It is planned to produce a report of these findings in monograph form, including maps.

Current analysis using these data is focused on a comparison adverse pregnancy outcomes in counties with significant chemical and petroleum manufacturing industry with counties of similar size and location, but without such industry. A report of these findings should be completed by FY 1980.

PROJECT NUMBER (Do NOT use this space)	HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01-HD-00305-02	EB

PERIOD COVERED

October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Effect of Illegitimacy on Infant Mortality From Linked Birth-Death Records in Minnesota, 1967-1973.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Carl A. Keller	Acting Chief	EB	NICHD
Other:	Karen Fetterly	Computer Specialist	CS/EBRP	NICHD

COOPERATING UNITS (if any)

LAB/BRANCH

Epidemiology Branch

SECTION

NICHD, NIH, Bethesda, MD

INSTITUTE AND LOCATION

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.05	.05	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Illegitimacy has often been implicated as a risk factor in infant mortality. An analysis of linked birth-death records from Minnesota which allows information from birth certificates to be related to infant death rates, has indicated that among very young women, in whom most illegitimacy occurs, marital status is not a risk factor for early neonatal mortality, but may be for post-neonatal deaths.

PHS-6040
(Rev. 10-76)

1. Project Description:

The data needed for this project have already been obtained through the LINK program and are part of Branch resources.

Analysis of the association between illegitimacy and early neonatal mortality has been completed and was presented at a national meeting in June, 1978.

Analysis of the association between illegitimacy and post-neonatal mortality was completed and presented in October, 1978.

Two manuscripts are currently being prepared.

This project will be completed by the end of FY 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD-00306-02 EB

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

The seasonality of Infant Mortality From Linked Birth-Death Records in North Carolina, 1967-1973.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I. :	Robert Nugent	Research Assistant	EB	NICHD
Other:	Ernest Harley	Computer Specialist	CS/EBRP	NICHD
	Carl A. Keller	Acting Chief	EB	NICHD

COOPERATING UNITS (if any)

LAB/BRANCH
Epidemiology Branch

SECTION
NICHD, NIH, Bethesda, MD

INSTITUTE AND LOCATION

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.10	.10	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The observed seasonality of neonatal deaths in the U.S. has implications as to the rule of environmental agents, e.g., infectious organisms, during pregnancy in influencing pregnancy outcomes. Since seasonality of conceptions may generate seasonality of neonatal death rates, linked birth-death records from North Carolina are being utilized to examine this relationship so that seasonality of death rates can be assessed independently of any seasonal variation in conceptions.

PHS-6040
(Rev. 10-76)

1. Project Description:

The data needed for this project have already been obtained through the LINK program and are part of Branch resources.

The neonatal mortality rate shows an excess of deaths during the first week of life each year in the late spring in the United States. An investigation of the nature of this peak is being made using linked birth-death records so that an account may be made for seasonal variations in conceptions, gestation length, and fetal deaths. Any seasonal variation in infant deaths not due to these factors has implications for variations in infant mortality from environmental causes such as infections.

Analysis is currently nearing completion, and results were presented in October, 1978 at a national meeting with a manuscript to be submitted by the end of calendar year 1979.

PROJECT NUMBER (Do NOT use this space)	HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01-HD-00307-02	EB

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Digital Preference of Conceptions in Missouri, 1968-1973

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I. :	Robert Nugent	Research Assistant	EB	NICHD
Other:	Carl A. Keller	Acting Chief	EB	NICHD
	Ernest Harley	Computer Specialist	CS/EBRP	NICHD

COOPERATING UNITS (if any)

LAB/BRANCH
Epidemiology Branch

SECTION
NICHD, NIH, Bethesda, MD

INSTITUTE AND LOCATION

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.40	.40	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

During the investigation of seasonal patterns of infant mortality, a conception cohort was constructed from birth certificate data. It was noted that there was a marked increase in the number of conceptions reported as occuring near the beginning of each calendar month. This has been verified as being due to digital preference for the 15th of the month when reporting the last menstual period. The possible consequences on infant mortality of errors through performance of elective procedures such as caesarian section is being investigated.

PHS-6040
(Rev. 10-76)

1. Project Description:

Data used for this project are based on LINK program as part of Branch resources.

During construction of a conception cohort for other purposes, it was noted that there was a marked digital preference in reported LMP date. The most commonly reported date was the 15th, followed closely by the 1st of the month. Other days which were siginficantly elevated were the 5th, 10th, 20th, 25th, and 28th. In addition, women who did not report an exact LMP date were compared to women who did. Women who did not report an LMP date tended to deliver a higher proportion of LBW babies. This difference was still seen after adjusting for maternal age, parity, legitimacy, and education. Studies on the difference in mortality rates between the two groups are anticipated and the results will be published when that data has been analyzed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD-00310-02 n EB Interagency Clinical Investigation #78-CH-102w/NIAMDD

PERIOD COVERED

October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Infant Feeding Among the Pima Indians: Time-trend, Factors Associated with Infant Feeding and Its Effect on Child Health

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I. :	Michele R. Forman	Senior Epidemiologist	EB/EBRP/NICHD
Others:	Ernest Harley	Computer Specialist	CS/EBRP/NICHD
	Norman Kretchmer	Director	NICHD
	Peter Bennett	Chief	EFB/NIAMDD
	Frank Falkner	Director	Fels Res.Inst.
	Michael Reed	Research Assistant	EB/EBRP/NICHD
	Robert Beren	Medical Records Abstractor	EB/EBRP/NICHD
	Kathleen Thomas	Secretary	EB/EBRP/NICHD

COOPERATING UNITS (if any)

NIAMDD
Fels Research Institute

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	.10

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The decrease in breast feeding in infants in the U.S. over the past 20 years has been reflected in Native American populations in a shorter period of time. This study is intended to document changes in breast feeding among the Pima Indians living on a reservation in Arizona and to compare the health effects of breast and bottle fed infants over the past 20 years. The availability of centralized health records at the Sacaton Hospital of the Indian Health Service and the relative lack of mobility of the population makes this an excellent source for the study of this issue of current national concern.

PHS-6040
(Rev. 10-76)

1. Project Description:

The objectives of this study are to determine the trend in breast and bottle feeding over time and the reasons for this trend among the Pima Indians and to determine whether breast feeding compared to bottle feeding is associated with reduced infections, reduced infant mortality, weight gains and cholesterol levels in children.

The methods being used to meet these objectives include interviewing a sample of 257 women on the Gila River Reservation in Arizona, reviewing medical records kept in the Indian Health Service Hospital, and locating and reviewing vital records on the study population. Development of the questionnaire, interviewer's and coder's manuals has been completed. The survey has also been computed and the analyses are being done at present. Review and abstraction of the medical records should be completed during FY 1979 as well.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979
Epidemiology Branch
Publications and Presentations

2. Publications:

Barr, M., Keller, C.A., Rogan, W.J., and Kline, J.: Summary of the workshop on perinatal and postnatal defects and neurologic abnormalities from chemical exposures. In Nicholson, W.J. and Moore, J.A. (Eds.): Health Effects of Halogenated Aromatic Hydrocarbons. New York, New York Academy of Sciences, 1979, pp. 458-472.

Nugent, R.P. and Keller, C.A.: Letter to the Editor: Errors in neonatal mortality statistics. Am. J. Obstet. Gynecol. 133:464-465, February, 1979.

Keller, C.A.: Epidemiological characteristics of pre-term births. In S. Friedman and M. Sigman (Eds.): Pre-term births: Relevance to Optimal Psychological Development. IN PRESS.

NICHD Annual Report

October 1, 1978 through September 30, 1979

Biometry Branch

The work of the Biometry Branch continued along three lines: (i) provision of statistical analysis and consulation to NICHD intramural and extramural investigators; (ii) pursuit of individual and collaborative research in biometry and biostatistics; and (iii) support of clinical trials initiated by the NICHD.

The Branch maintained its traditional strong ties to both the Intramural and Extramural programs of the Institute. A major commitment of staff time and effort continued to support follow-up and analysis of a randomized clinical trial of the safety and efficacy of phototherapy in comparison with exchange transfusion for the treatment of hyperbilirubinemia. Ms. Dolores A. Bryla and Mr. Nathaniel B. White, Jr. have devoted virtually all of their time to the analysis and data management of this clinical trial. The field phase of this project, funded by a contract in the Intramural Program and administered by the Scientific Director, NICHD, entered the fifth year of follow-up of children enrolled in the study. A comprehensive analysis of data relating to the treatment of newborns was completed in the past year, covering the following topics: deaths, metabolism, safety and efficacy of phototherapy, hemolytic disease, requirements for exchange transfusions, bilirubin, and effects on infants who are small for gestational age. An initial presentation of the study's findings was made at the May 1979 meeting of the Society for Pediatric Research, and a series of papers reporting the results will appear as a supplement to Pediatrics. Editing of the data from the first year follow-up examination is nearing completion, and analysis of this data will begin in the fall of 1979.

The results of the randomized clinical trial of vitamin C for the treatment of nephropathic cystinosis were reported in an April 1979 issue of the New England Journal of Medicine. Analyses relating to the natural history of cystinosis were completed for Dr. Joseph D. Schulman, Human Biochemical and Developmental Genetics Section, NICHD and Dr. Jerry A. Schneider, Department of Pediatrics, USC Medical School. Additional analysis and consultation is being provided to plan a possible future trial of cysteamine for the treatment of cystinosis.

Although the preceding two studies represented the major component of Biometry Branch support for intramural projects, additional consultations were made, a summary of several being as follows:

(i) A discriminant analysis of squirrel monkey vocalization data was performed for Drs. Harriet Smith and John Newman, Laboratory of Developmental Neurobiology, NICHD. The discriminant analysis was applied to a set of variables describing the "chuck" calls of five females living together in a social group. Using characteristics of the individual sound spectrograms, approximately 95 percent of all calls could be correctly assigned to the monkey making the call (as recorded by an observer during the pilot study). The discriminant functions resulting from the first phase of the study will be validated on a subsequent data set, and then this procedure will be used routinely to identify monkeys (without visual observation) in long-term studies of social interaction.

(ii) Analysis of daily urine measurements of LH and FSH taken during different stages of puberty was performed in collaboration with Dr. James Hansen, Neonatal and Pediatric Medicine Branch, NICHD. The investigation focused on the emergence, prior to menarche, of the maturing pattern of LH and FSH in response to the clinically evident increased level of estrogen produced by the ovaries. The results suggest that the typical adult positive and negative feedback relationships involving the gonadotropins (LH and FSH) and estrogen are established in sequential fashion during early pubertal development, Tanner stages I - III. Further studies are planned when the quantitative estrogen measurements become available.

(iii) An analysis of the effect of high dose vitamin E on various clinical parameters and the survival of red blood cells in patients with the Mediterranean variant of G-6-PD deficiency was performed for Dr. Joseph D. Schulman, Human Biochemical and Developmental Genetics Section, NICHD. The survival of red cells was extended by treatment, and the magnitude of the response to therapy was directly related to the severity of the disease. An extension of this study to neonates is being planned.

(iv) Methods of time series analysis and design issues in respect to the collection of serial serum determinations of pituitary hormones (LH, FSH, Prolactin) and gonadal hormones (testosterone, estradiol, dihydrotestosterone) from male subjects were discussed with Dr. George Merriam, Endocrinology and Reproduction Research Branch, NICHD. One result of the proposed studies would be to demonstrate or reject the notion that the episodic secretion of these hormones is linked to an "ultradian" rhythm, approximately 90 minutes in duration. If such a relationship could be established, it would be extremely helpful in quantifying the acute response to various treatments (for example, clomiphene citrate) when given to normal or hypogonadal men.

(v) Analysis of altered circadian rhythms for measurements of melatonin and cortisol in monkeys lesioned at the super-chiasmatic nucleus region of the brain was performed in collaboration with Dr. Steven Reppert, Laboratory of Developmental Neurobiology, NICHD, and Drs. Mark Perlow and Mort Mishkin, NIMH. Compared to sham-operated monkeys in which both melatonin and cortisol continued to cycle on a 24-hour schedule in both light-dark (LD) and all dark (DD) environments, the lesioned animals showed an uncoupling of the circadian rhythm of melatonin as compared to the 24-hour LD entrainment schedule. However, for the cortisol measurements, the 24-hour LD associated rhythm was abolished in only one of six lesioned animals. Methods of com-

plex demodulation and spectrum analysis were used to demonstrate the absence (or presence) of a phase-locked 24-hour rhythm in both the control and lesioned monkeys.

Work with NICHD staff in the Extramural Program ranged from participation in ad hoc contract review committees, to site visits, to serving as co-project officer on NICHD contracts. Biometry Branch staff have worked closely with scientists in the Center for Research for Mothers and Children (CRMC). Mr. Howard Hoffman in serving as co-project officer for the NICHD Cooperative Study of SIDS Risk Factors, funded by a contract with the Clinical Nutrition and Early Development Branch, NICHD. He serves as executive secretary to the Data Analysis and Methodology Advisory Committee for this study, is a member of the Steering Committee, and participates in frequent site-visits to monitor the project and advise the contractors on issues relating to the design, conduct and analysis of the study. Mr. Hoffman is also collaborating in a study of developmental neurophysiology and SIDS, funded by contract with CRMC. He is a co-editor of a book which will summarize the study findings, and is principal author of two chapters of the text, and is overseeing the multivariate analysis of potential risk factors for SIDS using the study data. Mr. Nathaniel B. White has been working with Dr. Thorsten Fjellstedt, Clinical Nutrition and Early Development Branch, Participating in site visits and reviewing progress reports and data analysis for the Human Milk Bank contract. Mr. White served as a reviewer of proposals submitted for a contract issued by the Human Learning and Behavior Branch, NICHD for the development of a prototype test of decoding skills which are pertinent to the assessment of dyslexia.

Consultation with staff in the Center for Population Research has been provided by Dr. James Schlesselman, who worked closely with scientists in the Contraceptive Development Branch and Contraceptive Evaluation Branch. Over the past year, he participated in site reviews of two projects funded by contracts with CPR. One was a randomized clinical trial evaluating use-effectiveness and method-effectiveness of two techniques of natural family planning (symptothermal vs. ovulation). The other was a study of the effects of induced abortion on subsequent pregnancy outcome. In addition he has collaborated with Dr. Richard Blye, Contraceptive Development Branch on the evaluation of the effectiveness of ethinyl estradiol and conjugated estrogens as post-coital contraceptives, and has worked closely with Dr. Bruce Stadel, Contraceptive Evaluation Branch, on the analysis of factors associated with variation in serum levels of ethinyl estradiol in healthy women who are taking the oral contraceptive.

Three contracts were administered by Biometry Branch staff. Work was completed on a contract with Boston University designed to study possible developmental neurophysiologic risk factors for the Sudden Infant Death Syndrome using a high risk population of twins (MZ and DZ pairs) and matched singletons. A monograph describing the study results will be available in the fall of 1979. Work was also completed on a contract established with the Mother and Child Institute, Warsaw, Poland under PL480 funds. An extensive final report which describes the effects of induced abortion on subsequent offspring has been prepared by the principal investigator, Dr. Halina Wior. A third contract, devoted to a study of the outcomes of

successive pregnancies in Norwegian women, completed the first year of work. Data supplied by the Norwegian Medical Birth Registry and by the Norwegian Central Bureau of Statistics were linked for a ten year period, 1967-1976, during the first contract year. A preliminary analysis of cause-specific mortality has been done.

Staff of the Biometry Branch frequently receive requests from outside investigators for statistical consultation. Branch policy allows consultative or collaborative relationships with non-NICHD investigators in order to enhance the influence and reputation of the NICHD. Mr. Howard Hoffman has consulted with Dr. Josephine Weatherall, Medical Statistician, Registrar Generals Office, England, on the analysis of data relating specific congenital malformations (e.g. neural tube defects, Downs Syndrome, and heart and circulatory system defects) to characteristics at birth, such as weight and gestational age. Mr. Peter Golblatt of the Office Of Population Censuses and Surveys, and Ms. Alison Macfarlane, National Perinatal Epidemiology Unit, Oxford, England, are producing the major tabulations for this study.

Mr. Hoffman has also worked with Dr. Glenn Bartlett, Hershey Medical Center, Pennsylvania, on developing bivariate contour plots of the frequency of live births and perinatal deaths in relation to sex and race of the newborn. He has collaborated with Dr. Don Gibson, National Institute on Aging, in establishing lifespan characteristics for rodents derived from NIA and NICHD program project and contract data. Mr. Hoffman participated in a contract review, funded by the Division of Research Resources, NIH, of the BIOMED computer package, and served on a site visit for an NIMH grant to study circadian rhythms and hormone measurements of humans placed in an environment which eliminates 24 hour diurnal time cues. He has also been called upon to discuss data related to the interruption of cyclic neutropenia in dogs after treatment with endotoxins. This arose as a consultation with Dr. David Dale and Dr. William Hammond of the University of Washington, Seattle. Mr. Hoffman has also advised investigators in Bar Harbor, Maine (Dr. David Harrison) and Oak Ridge, Tennessee (Dr. Michael Holland) on experimental design and data-analysis of lifespan data in rodents.

Dr. James Schlesselman was requested to prepare a paper for the Ethics Advisory Board, DHEW, on the assessment of the risk of abnormalities possibly resulting from the use of in vitro fertilization and embryo transfer in humans. He also participated in a site review of a study of child nutrition, vitamin A deficiency and xeropthalmia in Indonesia, supported by the Agency for International Development.

Details of the individual and collaborative research projects of Biometry Branch staff may be found in the project reports which follow this summary. Briefly, work was performed on the following topics: (i) writing a book on the design, conduct, and analysis of case-control studies; (ii) investigating the costs associated with several NICHD-funded case-control studies; (iii) characterizing the variation in serum levels of ethinyl estradiol in healthy oral contraceptive users; (iv) reviewing the epidemiologic literature pertinent to assessing potential risks from in vitro fertilization; (v) assessment of stimulation characteristics of intensive care

for infants with hyaline membrane disease; (vi) study of the tendency to repeat similar pregnancy outcomes in successive pregnancies; (vii) use of record linkage of U.S. states vital statistics to study maternal factors associated with infant mortality.

Other activities of Biometry Branch staff included serving as referees for papers submitted to the American Journal of Epidemiology, American Journal of Physiology, American Statistician, Biometrics, and Life Sciences. Mr. White served as co-chairman on the Committee on Minorities, American Statistical Association, and Mr. Hoffman was a member of a committee of the National Academy of Sciences advising on the use of animals in aging research.

Continued for the second year was a series of informal monthly seminars held by the Biometry Branch for staff in the Epidemiology and Biometry Research Program. Topics have been wide ranging, dealing with the analysis of data from clinical trials, laboratory investigations and epidemiologic studies.

NICHD Annual Report

October 1, 1978 through September 30, 1979

Biometry Branch

Publications and Talks

Publications:

Bakketeig, L.S., Hoffman, H.J. and Harley, E.E.: The tendency to repeat gestational age and birth weight in successive births. Amer. J. Obstet. Gynecol. (in press).

Harper, R.M., Walter, D.O., Leake, B., Hoffman, H.J., Sieck G.C., Sterman, M.B., Hoppenbrouwers, T. and Hodgman, J.: Development of sinus arrhythmia during sleeping and waking states in normal infants. Sleep 1: 33-48, 1978.

Perlow, M.J., Enna, S.J., O'Brien, P., Hoffman, H.J. and Wyatt, R.J.: Cerebrospinal fluid gamma-amino butyric acid: daily pattern and response to haloperiodl. J. Neurochem. 32: 265-268, 1979.

Hoffman, H.J.: Lifespan characteristics of selected inbred mouse strains, including F_1 and F_2 hybrids, and a four-way cross. Report of the Committee on Animal Models for Research on Aging, Subcommittee on Mice, National Academy of Science (in press).

Schlesselman, J.J.: The effect of errors of diagnosis and frequency of examination on reported rates of disease. Biometrics 33: 635-642, 1977 (omitted from Fy 78 report).

Schneider, J.A., Schlesselman, J.J., Mendoza, S.A., Orloff, S., Thoene, J.G., Kroll, W.A., Godfrey, A.D. and Schulman, J.D.: Ineffectiveness of ascorbic acid therapy in nephropathic cystinosis New Engl. J. Med. 300: 756-759, 1979.

Schlesselman, J.J.: How does one assess the risk of abnormalities from human in vitro fertilization? Amer. J. Obstet. Gynecol. (in press)

Talks

Bakketeig, L.S., Hoffman, H.J: Perinatal Survial Related to the Tendency to Repeat Gestational Age and Birth Weight in Successive Births. Seminar for the Departments of the Regius Professor of Medicine, and Social and Preventive Medicine, Oxford University, Oxford, England, October 10, 1978.

Hoffman, H.J.: Lifespan Comparisons for Selected Inbred Mouse Strains-Genetic and Environmental Factors. Biology of Aging Seminar, The Jackson Laboratory, Bar Harbor, Maine, January 3, 1979.

Hasselmeyer, E.G., Hoffman, H.J., Hunter, J.C.: Sudden Infant Death Syndrome and Vaccination for DPT: A Preliminary Report of Findings from the NICHD Cooperative Epidemiolgic Study of SIDS Risk Factors. Bureau of Biologics, Bethesda, Maryland, March 19, 1979.

Hoffman, H.J., Bakketeig, L.S.: Perinatal Mortality, Birth Order and Sibship Size. The Society for Pediatric Research Annual Meeting, Epidemiology Session, Atlanta, Georgia, May 3, 1979.

Hansen, J.W., Hoffman, H.J.: Urinary Gonadotropin Cycles in Prepubertal Girls. The American Pediatric Society, Endocrinology Session, Atlanta, Georgia, May 4, 1979.

Bakketeig, L.S., Hoffman, H.J.: Perinatal Mortality by Parity and Size of Sibship. Fifth Nordic Congress of Social Medicine, Helsingor, Denmark, June 14, 1979.

Schlesselman, J.J.: Use of Matching in the Design of Case Control Studies. American Public Health Association, Los Angeles, October 16, 1978

Schlesselman, J.J.: Assessment of Ascorbic Acid Therapy for Nephropathic Cystinosis. The Biometric Society, New Orleans, April 10, 1979

Bryla D.A., Scheidt, P. One-Year Follow-up of Infants Exposed to Ultrasound in Utero. Johns Hopkins University, Baltimore, Md. December 13, 1978.

White, N.B.,: The Epidemiology of RDS in the United States. USPHS Annual Meetings, Phoenix, Arizona, April 16, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-HD-850-03

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Controlled Study of Phototherapy for Neonatal Hyperbilirubinemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Dolores A. Bryla	Statistician	BB	NICHD
Karen L. Fetterly	Computer Sp.	EBRP	NICHD
Dorothy Herring	Statistician	BB	NICHD
Inez M. Roberts	Computer Sp.	EBRP	NICHD
Nathaniel B. White	Math. Statistician	BB	NICHD

COOPERATING UNITS (if any) Downstate Medical Center, State Univ., N.Y.
Albert Einstein College of Medicine
Long Island Jewish-Hillside Medical Center
Medical College of Virginia
Univ. Of Southern California Medical Center
Univ. of Cincinnati

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Md. 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.75	1.75	1.00

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study is a cooperative, randomized clinical trial to determine the safety and efficacy of phototherapy for treatment of neonatal hyperbilirubinemia by comparing phototherapy with non-phototherapy infants under specific conditions. Babies were randomized by weight (less than 2,000, 2,000 - 2,499 and greater than 2,499 grams) to the phototherapy or non-phototherapy groups. Infants 2,000 grams and above were admitted to the study when their bilirubin reached levels specified in the study protocol. All infants under 2,000 grams were admitted. Physical, neurological and mental development of these infants will be followed through six years of age.

PHS-6040
(Rev. 10-76)

Objectives: Determine short and long-term benefits and risks of phototherapy for treatment of newborn hyperbilirubinemia.

Methods Employed: This study is a cooperative, randomized clinical trial to determine the safety and efficacy of phototherapy for treatment to neonatal hyperbilirubinemia by comparing treated with untreated infants under specific conditions. The Biometry Branch serves as a data center for this study, and is the focal point for receipt of examinations 1,339 newborns and approximately 1,000 follow-up examinations each year until the child is six or seven years of age. These forms are checked for accuracy, and precoded in each center. The master files, 9,000 records containing newborn data, and 7,000 first year follow-up records, are edited for keypunch and coding errors, and for internal consistency. The Branch is also responsible for the analysis of the data.

Major Findings: Since the clinical trial is continuing, the final results are not yet available. An intensive analysis of the newborn data was begun in 1976 and the preliminary results were reported at the 1979 meeting of the American Pediatric Association and Society for Pediatric Research in Atlanta. The results will be published in a supplement to Pediatrics in the future. This supplement will include characteristics of the sample, analyses of the immediate safety and efficacy of phototherapy, exchange transfusions, hemolytic disease, metabolism, deaths, and small for gestation babies, and bilirubin-albumin binding. The one-year follow-up data which includes physical, neurological, ophthalmological, audiological and laboratory information is being edited. Analyses will begin in the fall ol 1979.

Significance to Biomedical Research and the Program of the Institute: There is a well documented relation between hperbilirubinemia during neonatal life and an increased risk of death or injury to the central nervous system from bilirubin encephalopathy, possibly resulting in impaired mental and neuromusclar abilities and deafness. Phototherapy has been widely accepted as a simple and effective treatment to reduce high bilirubin levels. In 1971, 51% of a sample of 300 U.S. hospitals were using phototherapy as a treatment procedure. In this study, phototherapy will be appraised for its immediate safety and efficacy, and for its possible long-term implications.

Proposed Course: To continue the analysis of the one-year follow-up data, with particular emphasis on the neurological and physical data. Follow-up examinations for second and third years will continue to be processed and analyzed by the Branch. The protocol and design of the sixth year follow-up examination forms will be developed in the fall of 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-HD-00801-04

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Studies based on the Medical Birth Registry of Norway, 1976-1973

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Howard Hoffman	Mathematical Statistician	BB	NICHD
Other:	May Chiu	Statistician	BB	NICHD
	Ernest Harley	Supervisory Computer Spec.	EBRP	NICHD
	Karen Fetterly	Computer Spec.	EBRP	NICHD
	Inez Roberts	Computer Spec.	EBRP	NICHD

COOPERATING UNITS (if any)

Institute of Hygiene and Social Medicine, University of Bergen, Norway
Department of Community Medicine, Univ. Of Trondheim, Norway

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	.5	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

These studies have focused on: (1) the relation of the quality of medical care to the risk of perinatal death in Norway, (2) the tendency to repeat similar birth weight and gestational age in subsequent pregnancy outcomes to the same mothers, and (3) perinatal mortality in relation to order of birth and size of sibship.

PHS-6040
(Rev. 10-76)

Methods Employed:
Methods used to analyze these data have included life-table techniques, contour diagrams, and various tabling and cross-tabulation procedures. Epidemiologic techniques have included "prospective" studies, case-control studies, and cohort studies.

Major Findings:
Several important findings have emerged from these studies. Norwegian births have been shown to be heavier and not as preterm as a comparable group of U.S. White births. A marked tendency for repeating low birth weight and/or preterm delivery has been demonstrated for successive pregnancies in this population of women. Current studies have explored the relation of perinatal mortality to birth order and size of sibship. As sibship size increases, perinatal mortality rates also increase. However, conditional on size of sibship, perinatal mortality rates decline as birth rank in sibship increases. The rates of low birth weight delivery ($\leq$2,500 gms.) follows the same patterm generally as that of perinatal mortality. This association may help account for the decline in perinatal mortality as birth rank increases. Birthweight specific perinatal mortality rates for mothers with sibships greater than two are approximately double (BW$\leq$2,500 grams) that for mothers with sibships of size one or two.

Significance to Biomedical Research and the Program of the Institute:
These studies have provided information on several issues pertinent to programs of the Institute. The "repeater" studies have helped to elucidate some of the etiologic problems of prematurity. Studies of fetal and infant mortality, and especially comparisons with available U.S. data sets, will aid understanding which of the cause-specific mortality categories the U.S. rates may be higher than Scandinavian rates.

Proposed Course:
Several studies have been published, others have been submitted for publication and still others are in the writing stage. The intent is to complete the studies which were started while Dr. Bakketeig was a Visiting Scientist at the NICHD.

Publications:

1. Bakketeig. L.S., Hoffman, H.J. and Harley, E.E.: The Tendency to Repeat Gestational Age and Birth Weight in Successive Births. Amer. J. Obstet. Gynecol. (in press)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD-00802-04

PERIOD COVERED

October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Study of Linked Information on Infant Death Certificates and Live Birth Certificates for Selected U.S. States

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Howard Hoffman	Mathematical Statistician	BB	NICHD
Other:	May Chiu	Statistician	BB	NICHD
	Ernest Harley	Supervisor Computer Sp.	EBRP	NICHD
	Karen Fetterly	Computer Sp.	EBRP	NICHD

COOPERATING UNITS (if any)

Departments of Health in the following states: California, Colorado, Minnesota, Missouri, New York, North Carolina, Rhode Island and Washington

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective is to assemble a multi-state data file of infant deaths in which prior linkage with birth certificate information has been performed. The studies to be done on the data set include associations between infant and fetal mortality with the standard information on birth certificates (e.g. birthweight, gestational age, maternal age and race, parity, etc). These studies will be compared with similar studies on a 1950 and 1960 cohort of U.S. births. Additional comparisions will be made to linked data from Canada (1971), Great Britain (1970) and Norway (1976-1973).

PHS-6040
(Rev. 10-76)

Methods Employed: Since linked data from birth certificates and infant death certificates is not available from the National Center for Health Statistics, we have dealt directly with individual State Centers for Health Statistics. We have chosen states which have routinely linked this information and are willing to cooperate in this venture. The data is received on magnetic tape from the individual states and merged into a common format. The job of editing these tapes and merging the information into common file is accomplished by the computer programmers within the Office of the Director, EBRP. When the study data sets have been edited and merged into a common file, the data will be analyzed using a variety of statistical techniques ranging from simple cross-tabulations to the making of sophisticated contour diagrams.

Major Findings: Perinatal mortality has been analyzed as a function of maternal age for birthweight-specific categories using 500-gram intervals. Maternal age has been stratified by single years through age 19, and by five year age groups thereafter. We have found that among white births, infants born to 19 year old mothers experience higher rates of "early neonatal mortality" (deaths ocurring in the first six days of life) compared to births of mothers aged 20-24 years, 25-29 years, 30-34 years for each 500-gram birthweight interval. Similarly, 18, 17, 16 and 15 year old mothers have successively higher rates of early neonatal mortality for each 500-gram birthweight category. Since these mortality rates are birthweight-specific, it is unlikely that the known association of preterm birth with younger maternal age can account for these results. Additional studies comparing fetal and stillbirth mortality rates by maternal age groups and birthweight-specific categories are underway.

Significance to Biomedical Research and the Program of the Institute: This study will provide a unique resource for the statistical and demographic study of infant mortality and some associated conditions (prematurity, teenage pregnancy, illegitimate births, etc.). Also, cause-specific infant mortality can be compared between the states participating in the study. The data will be available for the period 1968 through 1974 and will also permit an examination of time trends during this period.

Proposed Course: Data from Upstate New York have recently been received. Additional data from Missouri is being sought. Some analyses of these data will be used for an invited talk on fetal mortality rates at the Tenth Birth Defects Symposium in Albany, New York in October, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD-00806-02

PERIOD COVERED

October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Case-Control Studies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	James Schlesselman	Chief	BB	NICHD
	Daniel Denman	Statistician	BB	NICHD
Other:	Paul D. Stolley	Professor	Univ. Penn Medical School	

COOPERATING UNITS (if any)

University of Pennsylvania Medical School, Department of Research Medicine

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	.15	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A book on case-control studies is being written which will detail the logic of the study design, provide explicit recommendations for its application, and describe a variety of methods for the analysis of case-control data. The book is planned as a reference for researchers and advanced students in medicine, public health, epidemiology, and statistics.

PHS-6040
(Rev. 10-76)

Project Description and Progress to Date:

Drafts of eleven chapters, which shall comprise the entirety of the book, have been written, and a rewrite is in progress. Following is an outline of the chapters:

Outline

Preface

DES/Vaginal cancer

1. Introduction

    1.1 Distinction Between Experimental and Observational Studies (with examples); advantages and disadvantages of experimentation

    1.2 Observational Studies

        ecological study (defined; example)
        cohort study (defined; example)
        case-control study (defined; example)
        retrospective vs prospective cohort and case-control studies (defined; examples)

        Uses of case-control studies (exploratory vs analytic; advantages and disadvantages relative to cohort studies)

    1.3 Brief History of Case-control Studies (list major "discoveries" resulting from use of this method)

    1.4 Causation

        experimental criteria for causation
        observational criteria for causation

    1.5 Role of Statistical Analysis (p-values, etc.)

2. Measures of Risk

    2.1 Incidence
    2.2 Prevalence
    2.3 Statistical concept of association
    2.4 Measures of Association (relative risk, relative odds, risk difference, attributable risk)
    2.5 Cohort vs Case-control Paradigms in Relation to Sampling Frame and Estimates of Relative Odds
    2.6 Interaction (synergy/antagonism)

3. Case-Control Selection

   3.1 Case Definition (symptions; diagnostic criteria; misclassification; exclusions)
   3.2 Selection of Cases (representative sample)
   3.3 Sources of Cases
   3.4 Control Definition (exclusions)
   3.5 Selection of Controls
   3.6 Sources of Controls
   3.7 Examples of Case-Control Selection

4. Source of Bias and Problems of Confounding

   4.1 Bias in Determining Exposure
   4.2 Bias in Selection of Cases and controls
   4.3 Confounding
   4.4 Errors of Inference

5. Planning and Conducting a Case-Control Study

   5.1 Stating the Research Question
   5.2 Advance Planning of the Analysis
   5.3 Developing the research instrument
   5.4 Writing and Pilot Testing the Protocol
   5.5 Data Collection and Field Operations

6. Sample Size

   6.1 Introduction to the Determination of Sample Size
   6.2 Relative Numbers of Cases and Controls
   6.3 Sample Size for Stratified Studies
   6.4 Cost Considerations
   6.5 Power of Fixed Sample Size Studies
   6.6 Sequential Case-Control Studies
   6.7 Pair Matched Sample Size and Power

7. Matching

   7.1 Introduction (definition of terms and objectives of matching
   7.2 Matching Procedures
   7.3 Design Objectives (reduction of bias/increasing precision)
   7.4 Comparison of Matching with Other Adjustment Techniques
   7.5 Errors of Measurement
   7.6 Expected Number of Pairs Resulting from Postmatching

8. 8.1 2 x 2 Table (unmatched analysis)
   8.2 2 x 2 Table (matched analysis)
   8.3 Stratified Analysis (2 x 2 x t tables)
   8.4 2 x t Tables (trend in the odds of "disease")

9. Multivariate Analysis

   9.1 Multiple Logistic
   9.2 Logistic Analysis of Matched Pairs
   9.3 Loglinear Models

10. Point and Interval Estimation of Attributable Risk

   10.1 2 x 2 Table
   10.2 Adjustment for Confounding (2 x 2 x t table)
   10.3 2 x t Table (polytomous risk factors)

11. Controversies (detailed discussion of study design and interpretation in light of conflicting findings)

   11.1 Reserpine/Breast Cancer
   11.2 Postmenopausal Estrogens/Uterine Cancer
   11.3 Saccharin/Bladder Cancer

Significance to Biomedical Research and Program of the Institute: Case-control studies are extensively used to determine relatively rare adverse effects of drug or environmental exposure. For example, most of the adverse effects associated with use of oral contraceptives have been investigated using the case-control approach. Other current examples concern the discovery of an association between the development of vaginal cancer in young women with a previous maternal exposure to diethylstilbestrol during pregnancy, and studies of a potential link between bladder cancers and saccharin exposure in humans.

Proposed Course: Major publishers shall be contacted in the fall of 1979 in order to begin arrangements for completion of the book and its eventual publication. At the time each chapter has been rewritten, and exists in a form sufficiently "polished" to be circulated among colleages, it shall be distributed for comments and suggestions for improvements

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD-00 808-01

PERIOD COVERED

October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Variation of Ethinyl Estradiol Blood Levels among Healthy Women Using Oral Contraceptives

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Bruce V. Stadel	Medical Officer	CEB	NICHD
Other:	James J. Schlesselman	Chief	BB	NICHD
	Phyllis M. Sternthal	Statistician	BB	NICHD

COOPERATING UNITS (if any)

Contraceptive Evaluation Branch, NICHD

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.1	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Variation in serum levels of ethinyl estradiol was studied in 104 healthy women using oral contraceptives. Over 70% of the variation among women persists after the effects of the time interval between pill ingestion and blood drawing and the day of the menstrual cycle when blood was drawn are accounted for. This 70% residual variation is not accountable on the basis of age, height, weight, blood pressure, cigarette smoking, alchol use, or total lifetime OC use, although slight relationships between EE blood levels and some of these variables are suggested.

PHS-6040
(Rev. 10-76)

Methods Employed: One hundred four consenting OC users attending the Family Planning Clinic at Grady Memorial Hospital in Atlanta, Georgia, during the interval 1976 to 1978 were studied. Each subject was recruited and evaluated during the course of a single, routine Family Planning Clinic visit, provided that three criteria were met: 1) current combined oral contraceptive use of more than 30 days duration, 2) a diastolic blood pressure of less than 90 mm Hg upon routine screening during the clinic visit, and 3) no history of prior hypertension.

The following information was obtained from each subject: race, age, weight, height, systolic and diastolic blood pressure, brand and dosage of oral contraceptive currently in use, the number of hours since ingestion of last active pill (hereafter, "hours since pill"), lifetime oral contraceptive use (in months), day of menstrual cycle, cigarette smoking (average number per day), coffee consumption (average number of cups per day) and alcohol consumption (average number of bottles of liquor and wine, and bottles or cans of beer per week). Finally a venous blood specimen was obtained from subjects and ethinyl estradiol (EE) concentrations were measured using an established radioimmuno-assay procedure.

Major Findings: The average EE blood level was 88.3 pg/ml with a standard deviation of 39.7 pg/ml. The number of hours elapsed since ingestion of an oral contraceptive pill showed the strongest relationship with EE, accounting alone for 25% of the variation in EE levels among women. Together with day of cycle, these two variables explained 28% of the variation in EE.

Significance to Biomedical Research: Given the well established relationship between endogenous estrogens and the risk of breast cancer, and the growing concern that OC use may increase breast cancer risk for some women, it seems important that research concerning blood level responses to ingested contraceptive steroids be conducted. If some women do in fact exhibit consistently high blood level responses to steroid contraceptives, their optimum doses would be lower than those of women who respond with lower levels.

Proposed Course: A manuscript describing the study and its results has been written and submitted for publication.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD 00809-01

PERIOD COVERED

October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Assessment of the Risk of Abnormalities From Human In Vitro Fertilization

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: James J. Schlesselman Chief BB NICHD

COOPERATING UNITS (if any)

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A review of epidemiologic data relating to the risks of spontaneous abortions chromosomal abnormalities and congenital malformations was conducted to establish a baseline against which experience from pregnancies resulting from in vitro fertilization might be compared.

PHS-6040
(Rev. 10-76)

Method Employed: Review of published biomedical data.

Major Findings: In vivo, 40% to 50% of implantation blastocysts are estimated to have a chromosomal abnormality, over 99% of which are estimated to be eliminated during the course of pregnancy. In principle, the use of early spontaneous abortions can be more efficient than amniocentesis for detecting an increased risk of chromosomal abnormalities at birth. Unless *in vitro* fertilization in humans strongly contradicts the experience in domestic animal reproduction, which suggests no increased risk of abnormalities at birth, a large number of births would be required to provide a definitive assessment of risk.

Significance to Biomedical Research and the Program of the Institute: This review was prepared for the Ethics Advisory Board, DHEW, which was charged with the responsibility for determining whether restriction of federal funding should be removed for studies of *in vitro* fertilization. The NICHD is the leading biomedical agency which supports work relevant to the area.

Proposed Course: A revision of the manuscript was prepared for publication and is now in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD-00810-01

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Costs of Case-Control Studies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	James J. Schlesselman	Chief	BB	NICHD
Other:	Bruce V. Stadel	Medical Officer	CEB	NICHD
	Dorothy Herring	Statistician	BB	NICHD

COOPERATING UNITS (if any)

Contraceptive Evaluation Branch, NICHD

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.1	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A financial review of five case-control studies funded by the NICHD is being conducted to identify the costs associated with the planning, conduct and analysis of case-control studies. Expenditures associated with data collection, travel, operating expenses, and salaries of professional staff have been summarized for three phases of each study (1) planning, (2) data collection, and (3) analysis.

PHS-6040
(Rev. 10-76)

Method Employed: A systematic review of project protocols and financial reports, on file in the NICHD Contracts Office, has been done for five recently funded case-control studies.

Major Findings: Work is in a preliminary phase and consequently results are not yet available.

Significance to Biomedical Research and the Program of the Institute: Identification of costs associated with the planning, conduct and analysis of case-control studies will assist in the assessment of costs likely to be incurred in future studies funded by the Institute, and will provide a benchmark for gauging expenses associated with NICHD supported projects

Proposed Course: Analysis of accumulated data is underway, and a paper describing the results of the study will be written in FY 1980

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER [illegible]305-02

PERIOD COVERED

October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Stimulation Characteristics of the Intensive Care Environment of Hyaline Membrane Diseased Infants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Juarlyn L. Gaiter	Children's Hospital	
	Gordon B. Avery	Children's Hospital	
Other:	Nathaniel B. White	BB	NICHD

COOPERATING UNITS (if any)

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.05	.05	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The central purpose of the investigation is to observe the stimulation which a newborn with Hyaline Membrane Disease (HMD) receives in a hospital nursery in order to help understand what environmental influences impinge upon the sick premature. The impact of variations from the "normal" course of postnatal care such as treatment in an intensive care nursery is unknown. The current investigation (the pilot) systematically observes infants to obtain information regarding the content, frequency, and patterns of animate and inanimate stimulation experienced by HMD infants in a hospital setting. Observational data will be collected in order to document levels of auditory, visual, and caregiven stimulation received by the infants. Comparisons will be made with (a) other premature and (b) well babies in the larger follow-up study.

PHS-6040
(Rev. 10-76)

Objective: The specific objectives of the investigation are: (1) to develop an observation method suitable for describing the nursery environment, (2) to study a relatively homogenous sample of infants, i.e., hyaline membrane diseased (HMD) infants to obtain maximally meaningful information about the nature of their nursery experience, (3) to differentiate aspects of the nursery environment into conceptually meaningful dimensions of stimulation typically experienced by HMD infants, (4) to obtain data concerning the consistency and changes across time in the content/frequency and duration of stimulation HMD infants receive during intensive care, intermediate care and treatment one week prior to the expected date of discharge, (5) to obtain varied measures of infant developmental functioning for longitudinal follow-up studies of the infants, (6) to design appropriate interventions to facilitate the healthy development of HMD infants, and (7) to contrast animate and inanimate stimuli and observe their impact on the infants, (8) to compare the HMD babies with (a) other premature and (b) well babies.

Method Employed: A time sampling, observational system has been designed especially for use in recording stimulation data at the infant's bedside. Two trained observers, having obtained high inter-observer reliability, will code data on precoded observation sheets. One observer will record data on an individual infant subject during a given observation session and at subsequent sessions for the same infant. Each observer will record data on half (10) of the infant subjects in the pilot phase. A larger study will follow.

A total of six hours of observational data will be obtained for each infant. Two one-hour observations are designed to differentiate phases of medical treatment of HMD infants. These three differentiated treatment phases correspond to three types of environments in which caregiving-infant interaction are hypothesized to be measurably different. Measures of stimulation from the inanimate environment in the nursery will be obtained from sound and illumination level readings. The number of observations will be increased in the larger study.

Major Findings: A pilot study of 10 babies has been conducted and the data is being analyzed.

Significance to Biomedical Research and the Program of the Institute: Despite several published studies, we still do not have a clear delineation of the environment of the sick premature. There is an abuntant literature on the pathophysiology of hyaline membrane disease itself. Little has been written about the environmental impact of nursery care on the baby.

The present focus on manipulation of the environment of premature infants in lieu of obtaining detailed description of these environments threatens fruitful theorizing concerning early experience and its relationship to subsequent development.

The developmental significance of early differences in the environments of premature and term infants remains to be empirically determined. For this reason, it is necessary to conduct descriptive studies of these environments to generate testable hypotheses concerning their developmental impact. It is equally necessary to document infant behavior and responsiveness to stimulation as well as to identify increasingly complex behavior repertoires which assume stimulus value for caregivers. Besides providing information concerning the possible developmental effects of current nursery practice, observational, descriptive data may advance our understanding of factors which influence developmental outcome.

The proposed investigation is designed to develop an effective methodology for collecting stimulation data in the nursery. It will be important to examine a number of caregiver variables and attempt to replicate this investigation to determine if stimulation levels show consistency in this hospital nursery.

Proposed Course: A paper describing the pilot study is being prepared. The larger study has begun and all data should be collected by the end of 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-HD-00 804-04

PERIOD COVERED

October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Non-Parametric Tests of Spread

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Nathaniel B. White, Jr. Statistician BB NICHD

COOPERATING UNITS (if any)

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
:025	.025	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The central purpose of the investigation is to find a test statistic for the two sample dispersion problem which is: (1) not sensitive to the parent distributions of the sample points; (2) robust against differences in means and skewness; (3) comparable in power to other tests in the literature.

PHS-6040
(Rev. 10-76)

Objective: To find a two-sample test for differences in spread with the following properties: (1) freedom from assumptions about the shape of distributions (distribution free or at least robust), (2) freedom from assumption of equal location or at least relative insensitivity to moderate amounts of shift, (3) high mathematical power, (4) reasonable choice of tail probabilities when sample sizes are small, and (5) freedom from dependence on an arbitrary subdivision of samples into subsets.

Methods Employed: Monte Carlo evaluation of a number of alternative tests.

Significance to Biomedical Research and to the Program of the Institute: Tests could be useful in evaluating differences in dispersion in biomedical data where the assumption of normality is inappropriate.

Proposed Course: Results of earlier work are currently being summarized with hopes of expanding the work into a dissertation topic.

NICHD Annual Report

October 1, 1978 through September 30, 1979

Contract and Collaborative Research

Contract Title: The Epidemiology of Birthweight with Particular Reference to the Effect of Induced Abortion on Subsequent Offspring

Contractor: Mother and Child Institute, Warsaw, Poland

Money Allocated: 3,446,160 Zlotys (April 1, 1976 through March 30, 1978)

Objectives: To determine whether or not therapeutic abortion by means of dilation and curettage affects subsequent pregnancy outcomes.

Summary: The Mother and Child Institute supported by PL 480 funding has completed its study of the sequelae and complications of induced abortion by dilation and curettage. Approximately 11,000 pregnant women who registered in prenatal clinics for routine medical supervision were recruited into the study from Warsaw and Lodz. Case enrollment was completed in December 1976 and follow-up information on all subsequent pregnancies was finished in the fall of 1977. As a general summary of study findings, one can state that the interruption of a pregnancy, using dilation and curettage, had the following effects on a subsequent pregnancy: (i) increased risk of early bleeding, (ii) increased risk of spontaneous abortion in the second trimester, (iii) slightly increased risk of low birthweight. No effect of previous induced abortion on perinatal mortality was observed. Details of these findings are available in a final report prepared by Dr. Halina Wior, Mother and Child Institute.

Significance to Biomedical Research and Program of the Institute:
Therapeutic abortion is the predominant method of birth control in many countries, and has been increasing in frequency in the United States. The effect of abortion procedures on subsequent pregnancy outcome is a question of utmost importance which has yet to be adequately answered.

Proposed Course:
The final report is being prepared for possible publication, in extenso in English, and in a shortened Polish version, as a Research Report, edited by the Mother and Child Institute, Warsaw, Poland. Parts of the study are also being included in research reports of the WHO Collaborative Study (e.g. Lancet, January 20, 1979, pp 142-145).

Project Officer: James J. Schlesselman, Ph.D.

NICHD Annual Report

October 1, 1978 through September 30, 1979

Biometry Branch

Contract and Collaborative Research

Contract Title: A study of Monozygous and Dizygous Twins to Ascertain Possible Neurophysiologic Factors for the Sudden Infant Death Syndrome

Contractor: Boston University, Boston Massachusetts (N01-HD-3-2789)

Money Allocated: $206,232 (July 1, 1978 through August 31, 1979)

Objectives: One of the distinguishing characteristics of Sudden Infant Death Syndrom (SIDS) is that the peak age at death is between two and three months of age. This project is using a twin model to distinguish which of the potential risk factors for SIDS in the study are under ontogenetic control and which are not. Sleep apnea, cardio-pulmonary regulation and neurophysiologic organization (including sleep stage development) are being studied longitudinally in twins and in matched controls. From a variety of epidemiologic sources, twins have been shown to have about a three-fold increased risk of dying of SIDS compared to singletons. This apparent risk differential may be largely due to the generally earlier delivery and lower birthweight of twins. Thus birthweight, gestational and biometric growth parameters are being analyzed in relation to the physiologic and developmental variables in the study.

Major Finding: In addition to the sleep apnea results which have been published (Pediatrics 59:182-194, 1977), subsequent analysis has shown that:

1. Apnea is lowest in infants with poor somatic growth;
2. High apnea levels are not associated with poor central nervous system function as assessed by sleep state or sleep spindle maturation;
3. Apnea levels are not associated with motility levels; and
4. Colds are associated with a decrease in apnea levels during rapid-eye-movement and interdeterminate sleep, although not in quiet sleep.

Also, at 40 and 44 weeks of post-conceptual age, cardiac and respiratory control measures are correlated within sleep states. Neither cardiac nor control measures are correlated across sleep states, however. Thus abnormalities in physiologic control may be highly specific to sleep state.

Significance to Biomedical Research and Program of the Institute: Study results should provide evidence about the extent to which the various factors under study are under genetic or environmental control. As the variables under study have been implicated in SIDS etiology, study results should provide a rational basis for selecting variables which can be manipulated to prevent SIDS. If behavior (Sander) bed recordings reliably and validly register polygraphic activity, this device could provide relatively inexpensive screening for high SIDS-risk infants.

Proposed Course: This contract will expire on August 31, 1979.

Project Officer: Howard Hoffman

NICHD Annual Report
October 1, 1978 through September 30, 1979

Biometry Branch
Contract and Collaborative Research

Contract Title: Outcome of Successive Pregnancies for Norwegian Women 1967-1976

Contractor: University of Trondheim, Trondheim, Norway

Money Allocated: $20,463 (August 1, 1979 through July 31, 1980)

Objectives: This contract relies upon a unique data base to study some statistical aspects of repeating premature or mature births, in a well-defined national cohort of mothers. This study will make a valuable contribution to research aimed at elucidating factors that predispose to pre-term or low birth-weight delivery. Infant mortality will be studied as it relates to prematurity and the tendency to repeat similar gestational age and birthweight in subsequent deliveries to the same mother.

Major Findings: Preliminary data tabulations have focused on causes of death as a function of parity and birth weight ($\leq$1500, 1501-2500,>2500 grams). Earlier findings have shown that perinatal mortality increases as size of sibship increases, and that for a given size of sibship, perinatal mortality declines as birth order increases. The birthweight-specific cause of death data have shown that several particular causes of death are elevated for the higher risk low-parity-and-large-sibship births. As an example, deaths due to RDS are more common in this risk group. Also, deaths as a result of congenital anomaly are increased in this risk group. Several other causes of death do not occur more frequently than expected in this risk group. Most of the deaths are associated with a low weight birth. Since birth weight is a strong, independent risk factor, all of the comparisons apply to birth weight specific categories.

Significance to Biomedical Research and Program of the Institute: An important result of this study will be to identify specific causes of death which are implicated by various risk factors for perinatal death. In addition to the risk associated with low-parity-and-large-sibship births, this study will investigate the risks of repeated low birth weight and/or preterm delivery. This tendency to repeat premature delivery is one of the strongest predictive associations known from previous studies of the epidemiology of prematurity.

Proposed Course: Funding for an additional year is anticipated.

Project Officer: Howard Hoffman

NICHD ANNUAL REPORT
October 1, 1978 through June 30, 1979
Developmental Pharmacology Branch

SUMMARY

The DEVELOPMENTAL PHARMACOLOGY BRANCH studies the molecular mechanisms underlying genetic differences involved in the stimulation of pharmacologically important enzyme activities by drugs and various environmental contaminants and subsequent metabolism by these enzymes. Why does the same dose of the same drug or other compound given to two different individuals (with the exception of monozygotic twins) evoke different responses? The responses include therapeutic effectiveness of a drug and undesirable side effects such as tissue necrosis, cancer, and birth defects. Our studies are conducted with a variety of biological systems, each chosen so as to provide unique experimental access and unequivocal answers to the questions being asked. From results obtained with such experimental systems as inbred strains of mice and established cell culture lines, we can form hypotheses which can be quickly tested in clinical patients. The Branch presently consists of one Section and three Units.

A. The Section on Pharmacogenetics and Molecular Teratology, under the direction of Daniel W. Nebert, M.D., studies the relationship between the etiology of birth defects and the transplacental effects of environmental stimuli (drugs and other foreign chemicals) on such enzyme activities. In certain instances, numerous pharmacogenetic disorders in man cause individuals to react very differently to the same dose of the same drug. The teratogenic, carcinogenic, or toxic effects of certain drugs and other foreign compounds (xenobiotics) may also reflect important genetically mediated differences between individuals. Accordingly, our laboratory has developed experimental model systems for studying drug metabolism in cell culture and in a colony of inbred strains of mice. We have determined that the induction of at least 23 drug-metabolizing enzyme "activities" is regulated at one or two genetic loci, called the Ah locus for aromatic hydrocarbon responsiveness. We have developed an assay for the receptor which regulates induction of these enzyme activities and antibodies to several of the structural gene products (membrane-bound forms of cytochrome P-450) for use as probes to understand further the genetic regulation. The relative "aromatic hydrocarbon responsiveness" in certain mice results in more polycyclic hydrocarbon-initiated tumors, in a shortened paralysis time when given a certain muscle relaxant, in a shortened survival time when dosed with large amounts of polycyclic hydrocarbons, in hepatic necrosis and cataract formation when given acetaminophen, in an increased number of fetal resorptions, stillbirths, and malformations when the pregnant mother is given certain chemicals, and in ovarian toxicity. There now exists considerable evidence for the Ah locus in the human. These genetically mediated dissimilarities in xenobiotic metabolism may therefore be a useful probe in man for demonstrating differences in the rates of certain drug-induced birth defects.

B. The Unit on Genetic Regulation of Drug-conjugating Enzymes, under the direction of Ida S. Owens, Ph.D., is interested in genetic regulation of basal

and inducible glucuronide-conjugating enzyme activities among inbred strains of mice and in cell culture. Detoxification of certain highly lipophilic endogenous substrates and noxious foreign compounds, such as environmental pollutants, requires a multistep process involving phase I and II enzyme systems. With inbred strains of mice, research in this Branch has established that certain monooxygenases (phase I enzymes) are regulated at a few genetic loci and that expression of enzyme induction is dominant or codominant in response to aromatic hydrocarbon treatment. Since certain enzymatic products of the monooxygenase system are often toxic intermediates which can serve as substrates for conjugation (detoxification) via the UDP glucuronosyltransferase system (phase II enzymes), we are eager to determine the extent of coupling between these two enzyme systems in various tissues. We have shown that induction of several hepatic UDP glucuronosyltransferase activities by aromatic hydrocarbons is genetically linked to induction of the monooxygenase system. Furthermore, our studies concerning mutagenicity have shown that induced transferase activity can prohibit the further metabolism and conversion of hydroxylated metabolites of the monooxygenase system to mutagenic products. With bilirubin as substrate, the transferase in certain inbred strains (aromatic hydrocarbon responsive) of mice has two inducible peaks of activity while the transferase in other strains (aromatic hydrocarbon nonresponsive) has one peak of inducible activity. These patterns of inducibility in animal models indicate that in nature (perhaps in humans) more than one form of bilirubin UDP glucuronosyltransferase activity exist. More than one bilirubin transferase activity in humans is also suggested by the different modes of inheritance of defective transferase activity. A consideration of the metabolic pathway for foreign compounds shows that initially monooxygenase inserts a substituent group before one of several other drug-metabolizing enzymes can act on the compound either to toxify or detoxify the compound. Hence, an understanding of the functional relationship (i.e. balance) of each of these enzymes in various tissues might explain target tissue and carcinogenesis, toxicity, and mutagenesis of certain xenobiotics.

C. The Unit on Physical Biology, under the direction of Jack S. Cohen, Ph.D., examines the structure and interactions of biological molecules which have been probed in detail using several nuclear magnetic resonance spectroscopic methods. An alternating conformation has been shown to characterize the phosphodiester backbone of double-stranded poly(dAdT) in solution from the observation of a bifurcated ^{31}P NMR signal. This result may indicate the presence of sequence-specific local variations in the helical structure of DNA, which may have implications for protein-DNA interactions. The ^{31}P NMR technique has also been applied to measure the internal pH of cells. We have carried out the first titration of internal ATP, in chromaffin granules, and have shown that calibration methods for internal pH used until now have been inadequate. Using synthetic phospholipid vesicles we have recently extended this study to divalent metal ion effects on the titration of internal Pi. We have prepared the first singly deuterium-labeled protein, [ε -^{2}H]His 15 hen egg white lysozyme, and have used deuterium NMR to study its mobility and self-association in solution. Due to the particular magnetic properties of ^{2}H, this may be a general method for such measurements, and we are investigating the possibility of resolving signals from the protein residues attached directly to the iron atom in heme proteins. We have already used ^{13}C as another non-disturbing stable isotope probe to monitor the methionine methyl

group adjacent to the heme in cytochrome c, and are currently studying the conformation and ion-binding properties of this molecule. We have similarly used selective ^{13}C enrichment to study the binding of synthetic amino-terminal peptides of RNase in the RNase S system. In a recent unique application of this method we have quantitatively followed the peptide exchange process as a function of time and derived dissociation constants. We are now beginning a study of the binding of single- and double-stranded polynucleotides to RNase and histones using the above methods. (All of the above studies provide information on complex biological systems not readily amenable by other techniques).

D. The Unit on Genetic Expression, under the direction of Howard J. Eisen, M.D., is concerned with biochemical and molecular aspects of hormone action during fetal development. Fetal liver is used as a cellular model for analyzing the mechanism of glucocorticoid hormone action. The glucocorticoid receptor is purified by DNA-affinity chromatography, and anti-receptor antibodies are prepared for use in determining the chemical structure and biochemical function of the receptor. The role of glucocorticoids in controlling the development of carbohydrate metabolism in fetal liver is studied in utero and in vitro using a chemically defined organ culture system. The fetal liver organ culture system is also used to study the synthesis of insulin-like growth factors (somatomedins) at various stages of fetal development, and the synthesis of major secretory proteins (α-fetoprotein and albumin).

E. Clinical research protocols in this Branch include (i) kinetics of polychlorinated biphenyls concentration in breast milk during the first 6 months of lactation, (ii) attempts to develop a bio-assay for the determination of "breast milk jaundice factor," and (iii) family studies in an assessment of allelic differences at the Ah locus with: idiopathic aplastic anemia, solid tumors, childhood leukemia, and retinitis pigmentosa.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00032-06 DP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Structure and Function of Proteins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jack S. Cohen	Research Chemist	DP	NICHD
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LAB/BRANCH

Developmental Pharmacology Branch

SECTION

Unit on Physical Biology

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3/12	3/12	

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

To elucidate the conformation and interactions of proteins in solution, nuclear magnetic resonance (NMR) spectroscopy is the method of choice. To study strong and selective peptide-protein binding, as a model for hormone peptide-receptor interactions the ribonuclease S system has been chosen, since it provides a simple enzymatic test of biological viability. Selectively ^{13}C-enriched peptides of the (1-15) amino-terminal portion of ribonuclease were synthesized, and resolved resonances of individual ^{13}C atoms in the peptide-protein complex were monitored. Selective ^{13}C enrichment of methionine residues in cytochrome c have enabled us to monitor the conformational transitions of the protein and to show the existence of two distinct stable forms. Both at natural abundance (1.1%) and with ^{13}C enrichment the ^{13}C NMR technique has been extended to the observation of Met, His, Ala, Asp and Tyr residues in several proteins, and to the sugar component of glycoproteins.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To extend the study of functional groups (such as Met, Asp, Ala, etc.) in proteins by utilizing the intrinsic advantages of the NMR technique. Particularly it is intended to obtain unique information on specific atoms at a protein-peptide interface using the combination of NMR with selective stable isotopic enrichment. Similarly, conformational transitions and binding properties of heme proteins will be investigated.

Methods Employed: High field proton and carbon-13 nuclear magnetic resonance. Enrichment of biological molecules with stable isotopes (^{13}C, ^{15}N).

Major Findings:

A. Specific Peptide-Protein Interactions in the Ribonuclease S' System Studied by ^{13}C Nuclear Magnetic Resonance Spectroscopy: The results reported last year on the properties of the selectively ^{13}C enriched ribonuclease complexes are in the process of being published. Our most recent studies on these materials involves the measurement of the exchange rate (dissociation constant) of the peptide with the protein site in solution. This is achieved by following as a function of time either (a) the reduction of the ^{13}C NMR signal of the ^{13}C enriched peptide-protein (RNase S') complex, or (b) the decrease of the signal of the free ^{13}C-enriched peptide in the presence of unenriched complex. Since the naturally occurring RNase S-peptide is 20 amino acids in length and the synthetic peptide (giving full enzymatic activity) is only 15 amino acids long the values determined by these two methods are different. The K_d of the enriched complex was found to be $(0.2 \pm 0.8) \times 10^{-7}$ M at 4°C, and the value for native RNase S was ca. 2-3 times smaller. This method is analogous to radioactive tracer measurements, but has the advantage that it can be followed directly in solution without need to separate the components for measurements; it is a unique technique which may be generally applicable to quantitate peptide-protein interactions.

B. Monitoring the Carbohydrate component of the Fc Fragment of human IgG by ^{13}C Nuclear Magnetic Resonance Spectroscopy: The Fc fragment of human IgG is the first glycoprotein for which the three dimensional structure has been determined. The oligosaccharides are attached to asparagine residue 297 of the C_H2 domain. This domain is the one involved in the interaction between the antibody-antigen complex and the first component of the complement system. Therefore detailed structural information on it, particularly in solution, is of great interest. We have obtained ^{13}C nuclear magnetic resonance (NMR) spectra of the Fc fragment of human IgG1, which indicate the lack of independent motion of the carbohydrate moiety relative to the native protein. There are two broad bands observed in the region of the spectrum from 70-110 ppm. Resonances from the protein are not found in this region, but only resonances from the carbohydrate moiety. From the broadness of these bands, it would appear that the carbohydrate moiety is about as immobile as the protein itself. According to the X-ray determination of the Fc fragment structure, the carbohydrate moiety was shown to lie over a hydrophobic sequence of the Fc, and similarly in the structure of a complete IgG the carbohydrate was found to be buried. A very approximate estimate of the rotational correlation time for the carbohydrate component from the line widths is ca. $0.5\text{-}1 \times 10^{-8}$ sec. However, this is an average value of the many individual resonances which are

unresolved in this region of the native spectrum. In 6M urea this region becomes resolved into a number of narrow lines, which are characteristic of segmental flexibility. The urea causes the unfolding of the Fc fragment and the loosening of the carbohydrate group.

C. ^{13}C NMR Analysis of Methionine Sulfoxide in Protein: The $^{13}C^{\varepsilon}$ NMR signal of methionine sulfoxide is 22.6 ppm downfield from that of methionine. This affords the first method by which the extent of methionine oxidation can be determined in intact protein. We have demonstrated the utility of this approach with β-galactosidase enriched with ^{13}C in its methionine methyls.

D. The Anion Binding Sites of Transferrin: The iron transport protein transferrin has two specific metal-binding sites which are similar in their thermodynamic and spectroscopic properties. For each Fe^{3+} bound an anion must be bound, which is carbonate under physiological conditions. By employing ^{13}C enriched CO_3^{2-}, the anion binding can be studied by ^{13}C NMR. In ferric-transferrin, the ^{13}C resonance of CO_3^{2-} is broadened beyond detection due to its proximity to the paramagnetic Fe^{3+} ion, but the diamagnetic cobalt complex of transferrin reveals a distinctive resonance in the carbonyl region. Employing a mono-cobalt, mono-iron transferrin complex prepared by J. Zweier, the two metal-binding sites have been studied selectively in NMR experiments by J. Wooten. It is known that one carbonate is bound very tightly whereas the other exchanges slowly with free bicarbonate in solution. Since the characteristic CO_3^{2-} resonance was observed in the Fe-Co complex, it was concluded that the Co^{3+} ion occupies the non-exchanging site. The existance of non-specific anion binding sites was shown by the appearance of additional Co_3^{2-} resonances in the presence of excess bicarbonate. The resolution of the carbonate resonance into a doublet in a Co-Co complex suggests that two orientations or environments exist for the ligand in the Co^{3+} site.

E. Ferric Cytochrome c: The electron transfer protein in the mitochondrial respiratory chain, undergoes four pH-dependent conformational transitions as detected by optical spectroscopy. Each of these reflect perturbations of the electronic structure of the heme group. In addition, Morishima et al., have reported an additional conformational transition which was manifested by changes in the paramagnetically shifted resonances in the proton NMR spectra. Potentially more detailed information about these conformational states can be obtained from ^{13}C NMR due to its ability to resolve single carbon resonances at high magnetic field strengths at natural ^{13}C abundance and the ability to examine specific groups, in favorable cases, by ^{13}C enrichment. Work in this laboratory has made use of these two methods. Cytochrome c was prepared by Abel Schejter in which only the C^{ε}-methyl group of Met 80 was enriched to 45% ^{13}C. This allowed the examination of Met 80 in the native protein during the basic transition at pH 9. In this transition, Met 80 is removed as the sixth ligand of the heme. When the Met 80 is bound to the heme, the C^{ε}-methyl is not observable due to paramagnetic interaction with the unpaired heme electrons. As the pH is raised, Met 80 is displaced by Lys 79 and the resonance intensity grows. By varying the pH in small increments a titration curve was obtained for the intensity of the Met 80 methyl resonance. The shape of the curve appears to reflect the history of the sample. A sample purified on a Sephadex G-25 column gives a normal shaped curve with a Hill coefficient n = 0.9, but a sample prepared on a CM-cellulose column gave a curve with n = 0.75. The possibility that the two curves repre-

sent cytochrome in two different interconvertible conformational states is currently being investigated. The appearance of an additional resonance at neutral pH with slightly different chemical shift suggests the presence of a second conformational state in which Met 80 is displaced from the heme at lower pH, resulting in a more shallow pH titration curve ($n < 1$).

The natural abundance ^{13}C spectra observed also were sensitive to pH-dependent conformational states. Of particular interest is the collapse of a sharp singlet in the methylene region at pH 3 into numerous smaller lines at pH 5. This transition corresponds to the transition of pH 4 reported by Morishima which does result in alterations of the optical spectra. This transition may be due to the ionization of His 26 which is known to have a pK of 3.5 and appears to reflect the folding into the native physiological state from a partially unfolded state at pH 3. A comparative study of cytochromes from different species is underway in order to help assign individual resonances to particular residues.

Significance to Biomedical Research and the Program of the Institute: The results of this work and the development of the methods involved are of fundamental biochemical significance. Particularly, the detailed study of a model peptide-protein interaction is a first major step towards the much more difficult study of hormone- or drug-receptor interactions. Similarly the extension of the methods to study protein-nucleic acids could provide understanding of general significance.

Proposed Course of Project: (1) Further studies utilizing our unique ^{13}C-enriched peptides are continuing, particularly to develop the technique of stable isotope peptide-protein dissociation rate measurements, (2) the same RNase system will be used to study protein-nucleic acid interactions with both single and double standed oligodeoxy nucleotides, (3) studies of conformational effects and ion-binding to ^{13}C-Met cytochrome c will be finalized and will be extended to ^{2}H-substituted derivatives (see Project #00033-05 DP); the extension of these methods to other cytochromes will be investigated, particularly for CD_3-Met substitution, which may overcome problems of protein heterogeneity by concentrating on the functional groups, (4) the work on glycoproteins should be continued, but there is insufficient manpower for this, and (5) observations on stable serine-protease intermediates has had to be discontinued due to insufficient manpower.

Publications:

1. Shindo, H., Egan, W., and Cohen, J.S.: Studies of carboxyl groups in proteins by carbon-13 NMR spectroscopy. J. Biol. Chem. 253: 6751-6755, 1978.

2. Cohen, J.S., Yariv, J., Kalb (Gilboa), A.J., Jacobson, L., and Schechter, Y.: Carbon-13 NMR analysis of methionine sulphoxide in protein. J. Biochem. Biophys. Methods, in press, 1979.

3. Ohms, J.P., Hagenmaier, H., Hayes, M.B., and Cohen, J.S.: The near-heme histidine residues of deoxy- and oxymyoglobins. Biochemistry 18: 1559-1602, 1979.

4. Niu, C., Matsuura, S., Shindo, H., and Cohen, J.S.: Specific peptide-protein interactions in the ribonuclease S' system studied by ^{13}C nuclear magnetic resonance spectroscopy with selectively ^{13}C enriched peptides. J. Biol. Chem. 254: 3788-3796, 1979.

5. Rosen, P., Pecht, I., and Cohen, J.S.: Monitoring the carbohydrate of the Fc fragment of human IgG by ^{13}C NMR spectroscopy. Mol. Immunol. 16: 435-436, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00033-06 DP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Application of Stable Isotopes to Biochemical Problems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Developmental Pharmacology Branch

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5/12	5/12	

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose is to develop applications of stable isotopes (^{13}C, ^{15}N, ^{2}H) as non-disturbing probes in biochemical systems with the application of appropriate techniques (NMR, GC-MS). In order to accomplish this objective the synthesis of molecules containing these isotopes enriched at selected positions is undertaken, notably synthesis of peptides containing ^{13}C and ^{15}N enriched amino acids. Carbon-13 or nitrogen-15 NMR is used to study the structure and interactions with protein binding sites of such peptides in solution, utilizing the special advantages that such enrichment provides. Selectively deuterium-labelled molecules are also prepared to take advantage of the special properties of this isotope.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To develop new tools to probe molecular structure and function by utilizing the combination of stable isotopic substitution (^{13}C, ^{15}N, ^{2}H, ^{77}Se) with NMR observation of the isotopic probe.

Methods Employed: Nuclear magnetic resonance observation of the following nuclei: ^{13}C, ^{15}N, ^{2}H and ^{77}Se. A probe and appropriate radiofrequency units for our superconducting magnet system has been constructed to observe each of these nuclei.

Major Findings:

A. Protein Mobility and Self-Association by Deuterium NMR: Hen egg white lysozyme has been prepared in which the C^{ε} position of the single histidine residue is substituted by a deuterium atom as a non-disturbing stable isotope probe. This is the first report of a single ^{2}H labelled protein. The ^{2}H NMR spectrum in H_2O shows a broad resonance (500-1000 Hz) due to the histidine deuteron and a sharp signal from residual HOD. The line width of the deuterium signal increases with pH, reflecting the self-association of lysozyme which is known to involve this histidine residue (H. Shindo, J.S. Cohen, J.A. Rupley, Biochemistry 16: 3879, 1977). Correlation times calculated from spin-spin relaxation times (T_2) derived from the ^{2}H line widths indicate that (a) His 15 is restricted in motion in the protein, and (b) that lysozyme is predominantly dimerized at pH 7.5. Controls carried out with [ε-^{2}H] imidazole showed a small pH dependence of the spin-lattice relaxation time (T_1), which parallels the ^{2}H chemical shift change upon ionization of the imidazole. Similar results cannot generally be observed by ^{1}H NMR because of paramagnetic relaxation due to trace metal ion impurities. The pH dependence of the ^{2}H T_1's indicates a change in the ^{2}H quadrupole coupling constant upon protonation of the imidazole ring.

B. Cross-Peptide Bond ^{13}C-^{15}N Coupling Constants by ^{13}C and J Cross-polarization ^{15}N NMR: Comparative ^{13}C-^{15}N coupling constants have been determined for the linear dipeptide tBoc-L-[U-^{13}C]Ala-[^{15}N]GlyOMe and the corresponding cyclic diketopiperazine, both in dimethylsulfoxide (DMSO) and, upon removal of the tBoc group, in water solutions. Spectra were obtained by ^{13}C NMR and by the first application of J cross-polarization (JCP) ^{15}N NMR, which greatly reduces the time required to accumulate ^{15}N NMR spectra. In DMSO there was evidence for the formation of complexed species which were not present in water. The values obtained for the cross-peptide bond coupling constant $^{2}J_{13C\alpha_15N}$ were consistently less (by 2.2 Hz in DMSO, 4.3 Hz in water) for the cyclic than for the linear peptide, which may be related to the cross-peptide bond conformation. The ^{15}N resonance for the cyclic peptide was shifted only 2 ppm downfield from the linear peptide chemical shift value in both solvents.

Attempts to extend the ^{15}N NMR technique to ^{15}N-Ala 6 RNase (1-15) peptides were unsuccessful due to the increased signal line width.

Significance to Biomedical Research and the Program of the Institute: (1) Interactions and conformations of macromolecules in solution can be studied by relatively few techniques. It is important to develop new techniques

towards this end in order to obtain fundamental information about macromolecular function. (2) Use of stable isotopes in metabolic studies could be of great significance to neonatal systems.

Proposed Course: (1) The deuterium NMR method will be extended to protein-nucleic acid and other macromolecular interactions, and to investigations of iron-bound groups (Met, His) in heme proteins. (2) Extension of the ^{13}C and ^{15}N peptide studies is impossible with present manpower. (3) An attempt will be made to assess the utility of ^{13}C-enriched glucose as a metabolic probe.

Publications:

1. Cohen, J.S.: Nuclear magnetic resonance studies of biologically relevant isotopes other than hydrogen. Crit. Rev. Biochem. 5: 25-43, 1978.

2. Niu, C., Bertrand, R., Shindo, H., and Cohen, J.S.: Cross-peptide bond ^{13}C-^{15}N coupling constants by ^{13}C and J cross-polarization ^{15}N NMR. J. Biochem. Biophys. Methods, in press, 1979.

3. Wooten, J., and Cohen, J.S.: Protein mobility and self-association by deuterium NMR. Biochemistry, in press, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD-00136-11 DP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

PHARMACOGENETICS

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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91/12	64/12	27/12

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) In certain instances numerous pharmacogenetic disorders in man cause individuals to react very differently to the same dose of the same drug. The teratogenic, carcinogenic, or toxic effects of certain drugs and other foreign compounds (xenobiotics) may also reflect important genetically mediated differences between individuals. Accordingly, our laboratory has developed experimental model systems for studying drug metabolism in cell culture and in a colony of inbred strains of mice. We have determined that the induction of at least 23 drug-metabolizing enzyme "activities" is regulated at one or two genetic loci, called the Ah locus for aromatic hydrocarbon responsiveness. The relative presence of "aromatic hydrocarbon responsiveness" in certain mice results in more polycyclic hydrocarbon-initiated tumors, in a shortened paralysis time when given a certain muscle relaxant, in a shortened survival time when dosed with large amounts of polycyclic hydrocarbons, in hepatic necrosis and cataract formation when given acetaminophen, and in an increased number of fetal resorptions, stillbirths, and malformations when the pregnant mother is given certain chemicals. These genetically mediated dissimilarities in xenobiotic metabolism may be a useful probe in demonstrating differences in the rates of certain drug-induced birth defects, other forms of drug toxicity, and tumors.

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Project Description:

Objectives: Membrane-bound mixed-function oxygenases are important in the metabolism of drugs, carcinogens, and insecticides, as well as many endogenous lipophilic substrates such as steroids, fatty acids, cholesterol, indoles, biogenic amines, and thyroxine. We have found that the "induction," or stimulation, of a group of drug-metabolizing enzyme activities in response to aromatic hydrocarbon treatment is regulated by only a few genetic loci in the mouse. We have also employed cell culture techniques for studying the mechanism of microsomal monooxygenase induction in an experimental system which is more rigidly controlled than similar studies in the intact animal. Understanding the mechanism by which drug-metabolizing enzymes are induced may elucidate important concepts in the fields of drug metabolism, developmental pharmacology, steroid biochemistry, chemical carcinogenesis, teratology, entomology, and limnology. The main objectives of this project are (1) to exploit genetic differences in drug metabolism between various inbred strains of mice in order to understand better the potential importance of pharmacogenetics; (2) to use tissue culture techniques in answering questions which would be difficult or impossible with studies in the intact animal; (3) to combine the genetic differences with the tissue culture techniques in order to elucidate certain mechanisms related to mammalian gene regulation; and (4) to apply certain of these techniques to human tissues so that extrapolations can be made from rigidly controlled experimental systems to pharmacogenetic differences in man.

Methods Employed: The principal methods employed are: (1) recording spectrophotometry of turbid solutions, (2) enzyme assays involving radiometric, spectrophotometric, and spectrophotofluorometric determinations of product formation or substrate disappearance, (3) radioisotopic measurements using tritium and carbon-14, (4) cell culture techniques, (5) differential centrifugation, (6) phase microscopy, (7) polyacrylamide gel electrophoresis, (8) isoelectric-focusing electrophoresis, (9) column- and thin-layer chromatography, (10) magnetic resonance techniques, (11) high pressure liquid chromatography, (12) gas chromatography, (13) gas chromatography-mass spectrometry, (14) *in vitro* translation of mRNA, (15) peptide mapping *via* partial proteolysis, and (16) DNA recombinant techniques.

Major Findings: (1) In the liver of perinatal rats or mice, the ratio of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced aryl hydrocarbon hydroxylase to total cytochrome P-450 content decreases, whereas the ratio of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced acetanilide 4-hydroxylase to total cytochrome P-450 content increases, between 18 or 19 days and 22 days following conception. The ontogenesis of inducible aryl hydrocarbon hydroxylase corresponds well with increases in a 56,000-M_r electrophoretic band. The later temporal expression of inducible acetanilide 4-hydroxylase closely parallels 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced increases in size of a 54,000-M_r electrophoretic band and a 2-3-nm hypsochromic shift in the Soret peak of the total microsomal reduced cytochrome P-450 CO complex. The data provide evidence in the rodent for temporal control of the expression of different structural gene products regulated by the *Ah* locus.

(2) Allelic differences at the Ah locus are shown to exist in mouse brain. This finding probably explains inbred mouse strain differences in polycyclic hydrocarbon tumorigenesis of the brain described more than 35 years ago and may be important in understanding the etiology of genetic differences in certain human intracranial neoplasms.

(3) The in vitro DNA binding of benzo[a]pyrene metabolites generated by mouse liver microsomes can be resolved into at least nine distinct peaks by elution of a Sephadex LH20 column with a water-methanol gradient. These peaks, representing metabolite-nucleoside complexes, are named A (most polar) through I (least polar). The use of various microsomal enzyme inducers or inhibitors--combined with the underlying genetic predisposition of the individual, tissue, or cell culture system under study--demonstrates that the balance between P-450 and epoxide hydrase, and the ratio of each form of P-450 to the other forms of P-450, can influence markedly the quantity and quality of reactive intermediates of benzo[a]pyrene that bind to DNA.

(4) When [^{3}H]benzo[a]pyrene is incubated in vitro together with DNA, NADPH, and rat lung microsomes, covalent binding of benzo[a]pyrene metabolites to DNA occurs. 3-Methylcholanthrene pretreatment of animals induces the total covalent binding in vitro several-fold and increases the amounts of at least five metabolite-nucleoside complexes associated with the 7,8-diol-9,10-epoxides, the 7,8-oxide or quinones oxygenated further, the 4,5-oxide, and phenols metabolized further. These increases correspond well with the increases in the production of both non-K-region and K-region metabolites of benzo[a]pyrene by lung microsomes, as determined by high-pressure liquid chromatography. On the other hand, when [^{3}H]benzo[a]pyrene is metabolized in isolated perfused rat lung, only the peak representing the 7,8-diol-9,10-epoxide bound to nucleoside(s) is readily detectable and then only in lungs from 3-methylcholanthrene-treated animals. It is suggested that the further metabolism of primary benzo[a]pyrene metabolites in the intact tissue (e.g. conjugation reactions) is responsible for the differences in the metabolite-nucleoside patterns observed in vivo, as compared with microsomal metabolism in vitro.

(5) The formation of benzo[a]pyrene metabolite-nucleoside adducts in perfused rat and mouse liver and in mouse lung slices was studied by Sephadex LH20 chromatography. The results suggest that some of the benzo[a]pyrene metabolite-nucleoside complexes generated by microsomes and deproteinized DNA in vitro also occur in the intact rodent liver and lung tissues. Furthermore, complexes with the diol-epoxide(s) were observed with RNA from perfused liver of β-naphthoflavone-treated, but not from untreated or phenobarbital-treated rats. Complexes between ribonucleoside(s) and the diol-epoxide(s) were also found in perfused liver or lung slices from genetically responsive but not from genetically nonresponsive mice.

(6) Aryl hydrocarbon hydroxylase activity and the binding of benzo[a]pyrene metabolites to deproteinized DNA in vitro are both about 3 to 4 times higher in trout liver microsomes than in control rat liver microsomes. The hydroxylase activity and the DNA binding with liver microsomes from 3-methylcholanthrene treated rats are about 10 and 36 times, respectively, greater than in control rat liver microsomes. The liver microsomes from another fresh-water fish (roach) are relatively inactive, the enzyme activity about 7% and the DNA binding 11% of that in control rat liver microsomes. Several benzo[a]pyrene metabolite-nucleoside complexes were resolved with a Sephadex LH20 column

chromatography. These results show that the formation of different reactive benzo[a]pyrene intermediates which bind covalently to DNA is very similar between 3-methylcholanthrene-treated rat and untreated trout liver microsomes.

(7) The carcinogenesis index of subcutaneous benzo[a]pyrene-initiated fibrosarcomas in the genetically "responsive" C3H inbred mouse strain is more than five times higher, and about 15 times higher, than that in the "responsive" C57BL/6 and the "nonresponsive" DBA/2 strains, respectively. Carcinogenesis indices involving F_1 hybrids of these strains indicate that additional genes besides the Ah locus may cause a particular inbred strain to be more sensitive, or resistant, to benzo[a]pyrene-initiated tumors than would be expected solely on the basis of aryl hydrocarbon hydroxylase inducibility. Our data demonstrate a strikingly good correlation between genetically determined increases in peaks representing P_1-450 catalyzed benzo[a]pyrene metabolites bound to DNA and benzo[a]pyrene mutagenesis *in vitro*. Neither of these *in vitro* parameters, nor DNA repair in mouse skin *in vivo*, however, explains the 5- to 6-fold difference in benzo[a]pyrene carcinogenesis index between the two genetically responsive strains, C3H and C57BL/6.

(8) The binding of reactive benzo[a]pyrene metabolites to deproteinized DNA *in vitro* can be drastically changed, both quantitatively and qualitatively, *in vitro* by changes in the substrate concentration or the substrate/P-450 ratio, and in the intact animal by starvation or substitution of a "purified protein test diet" for the regular laboratory chow. Data from liver, lung, and bowel microsomes from C57BL/6N and DBA/2N mice demonstrate the importance of dietary contaminants or nutrition during benzo[a]pyrene tumorigenesis.

(9) The profile of DNA binding of benzo[a]pyrene metabolites was shown to be an extremely sensitive test for detecting minute amounts of induced cytochrome P_1-450 and its associated aryl hydrocarbon hydroxylase activity. A direct correlation is not necessarily observed, however, between "aryl hydrocarbon hydroxylase activity" and the amount of benzo[a]pyrene metabolites bound covalently to DNA. "Untreated" genetically responsive mice are shown to have greater "control" levels of benzo[a]pyrene metabolism in their various tissues than genetically nonresponsive mice simply on the basis that responsive mice have a lower threshold for "responsiveness" to exogenous inducers inhaled and/or ingested in their crude diet.

(10) Benzo[a]pyrene, at doses between 50 and 300 mg per kg body weight given at Day 7 or 10 of gestation, causes *in utero* toxicity and teratogenicity more so in genetically "responsive" C57BL/6 than in "nonresponsive" AKR inbred mice. With the use of AKR x (C57BL/6)(AKR) and (C57BL/6)(AKR) x AKR backcrosses, it was shown that allelic differences at the Ah locus in the fetus can be correlated with dysmorphogenesis. If the mother is nonresponsive (Ah^d/Ah^d), the Ah^b/Ah^d genotype in the fetus is associated with more stillborns and resorptions, decreased fetal weight, increased congenital anomalies, and enhanced P_1-450-mediated covalent binding of benzo[a]pyrene metabolites to fetal protein and DNA, when compared with the Ah^d/Ah^d genotype in the fetus from the same uterus. If the mother is responsive (Ah^b/Ah^d), however, none of these parameters can be distinguished between Ah^b/Ah^d and Ah^d/Ah^d individuals in the same uterus, presumably because enhanced benzo[a]pyrene metabolism in maternal tissues and placenta cancels out these differences between individual fetuses.

(11) Of particular interest in our study is the fact that the mother and the father both must be of a particular genotype before differences in teratogenesis among fetuses (due to their genotype) will be expressed. These data might provide an example in attempting to explain clinically why only one child is affected with an apparent "drug-induced syndrome" although the mother has taken the same dose of the particular drug during each of numerous pregnancies.

(12) The metabolism of various carcinogens and other chemicals by genetically regulated increases in cytochrome P_1-450 content was studied with the use of liver from 3-methylcholanthrene-treated C57BL/6N and DBA/2N mice. Genetic differences were seen in the DNA binding of metabolites *in vitro* for: dibenz[a,h]anthracene, benz[a]anthracene, benzo[a]pyrene, 3-methylcholanthrene, 7,12-dimethylbenz[a]anthracene, 2-acetylaminofluorene, and dopamine (approximately in descending order). Any difference for dibenz[a,c]anthracene or benzidine was very small. Marked genetic differences were observed in the mutagenicity (with *Salmonella typhimurium* tester strains TA1538, TA98, or TA100) *in vitro* for: 6-aminochrysene, dibenz[a,h]anthracene, dibenz[a,c]anthracene, β-naphthylamine, 3-methylcholanthrene, 2-acetylaminofluorene, benzo[a]pyrene, benz[a]anthracene, and α-naphthylamine (approximately in descending order); the difference for 7,12-dimethylbenz[a]anthracene was very small. Important discrepancies for certain carcinogens therefore exist between genetic differences in metabolites binding to DNA and genetic differences in the metabolites causing mutation in this bacterial *in vitro* assay. These data may be useful for future studies with any of these chemicals in the extrapolation of genetic differences observed *in vitro* to differences in the risk of cancer associated with the *Ah* locus in the intact animal.

(13) At oral benzo[a]pyrene doses estimated to be between 6 and 12 mg per kg body weight per day, leukemia develops 100 or more days later in the nonresponsive Ah^d/Ah^d homozygous mouse but not in the responsive Ah^b/Ah^d heterozygote. These results may be explained on the basis of "first-pass elimination" kinetics. An excess of dietary α-naphthoflavone 20 times greater than the amount of benzo[a]pyrene prevents these hematopoietic neoplasms. These data suggest that α-naphthoflavone-sensitive metabolism of benzo[a]pyrene--presumably cytochrome P_1-450 in the bone marrow of Ah^d/Ah^d individuals--is responsible for causing the leukemia.

(14) The induction of benzo[a]pyrene metabolism in the pigmented epithelium of the retina is associated with the Ah^b allele in the mouse. With the use of histofluorescent techniques and chick embryonic pigmented epithelium in tissue culture, we show that the monooxygenation of benzo[a]pyrene and the glucuronidation of *p*-nitrophenol (and most likely the metabolism of many other xenobiotics) is localized principally in the endoplasmic reticulum of retinal pigmented epithelium. These drug-metabolizing systems no doubt play an important role in both detoxification and metabolic activation of any foreign chemical entering the uveal circulation and reaching the photoreceptor cells through the choriocapillaris.

(15) Large doses of intraperitoneal acetaminophen administered to 3-methylcholanthrene-treated genetically responsive (Ah^b/Ah^b) inbred mouse strains and the responsive Ah^b/Ah^d heterozygote from the $(C57BL/6)(DBA/2)F_1$ x DBA/2 backcross cause opacity of the anterior portion of the lens to develop irreversibly within 6 hours. Such cataract formation does not occur in similarly treated nonresponsive inbred strains or nonresponsive Ah^d/Ah^d individuals from the

same backcross. Differences in acetaminophen metabolism and toxicity are known to be associated with the Ah locus in the mouse, and differences in heritability at the Ah locus are known to exist in the human. Our ophthalmologic findings therefore may be important clinically to certain patients receiving either a single large overdose or high chronic doses of this drug.

(16) 2,3,7,8-Tetrachlorodibenzo-p-dioxin is metabolized by the mouse liver cytochrome P-450-mediated monooxygenase system to reactive intermediates which bind "covalently" to cellular macromolecules. Although very difficult to quantitate, the presumably covalent binding to microsomal protein occurs between 120 and 2,640 times more readily than binding to deproteinized DNA in the in vitro reaction. Because of the extremely high rate of binding to protein rather than to DNA, it is visualized that 2,3,7,8-tetrachlorodibenzo-p-dioxin metabolites may be so reactive that they bind in or near the P-450 active-site where the 2,3,7,8-tetrachlorodibenzo-p-dioxin is monooxygenated. This extreme reactivity may preclude the formation of detectable quantities of phenols, dihydrodiols, or conjugated products. The rate of 2,3,7,8-tetrachlorodibenzo-p-dioxin metabolism is estimated to be between 9,000 and 36,000 times lower than the rate of P-450-mediated benzo[a]pyrene metabolism. These data may be important in explaining the extreme toxicity and teratogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin.

(17) Hexane extracts of four commercial preparations of coal tar shampoos were studied for their mutagenic properties in the Salmonella/liver test system in vitro. Three of the four shampoos were highly mutagenic, whereas the fourth was not--under our experimental conditions. By high-performance liquid chromatographic, gas-liquid chromatographic, and gas-liquid chromatography-mass spectrometric analyses, more than 35 distinct fractions could be resolved; seven polycyclic aromatic chemicals believed to be present in coal tar were tentatively assigned as the major component of some of these fractions. The shampoo extract that was most mutagenic had a greater number of distinct fractions and contained approximately 50 times more benzo[a]pyrene, compared with the one shampoo extract that was not mutagenic under our experimental conditions.

(18) A simple and very sensitive method for the separation of 4-hydroxyacetanilide, 3-hydroxyacetanilide, 2-hydroxyacetanilide, and acetanilide was developed with the use of high-pressure liquid chromatography. Each of these phenolic derivatives can be separated completely from acetanilide and from one another. A simple assay for "acetanilide 4-hydroxylase activity" is thus described. The limit of sensitivity for cytochrome P-450-mediated acetanilide 4-hydroxylase activity is estimated to be 1.0 pmol/min/mg microsomal protein, thereby allowing this assay to be useful in detecting monooxygenase activity in "low level" nonhepatic tissues.

(19) Hepatic acetanilide 4-hydroxylase activity is induced about 4-fold in C57BL/6N mice by 3-methylcholanthrene. The K_m values of both the 3-methylcholanthrene-induced and control 4-hydroxylase activity are about 0.55 mM; V_{max} values for 3-methylcholanthrene-treated and control mice, respectively, are 4.9 ± 1.1 and 1.1 ± 0.31 nmol/min/mg microsomal protein. The 4-hydroxylase in the liver of both 3-methylcholanthrene-treated and control mice appears to represent two or more catalytic activities, i.e. two or more forms of P-450 having widely differing affinities for the substrate acetanilide.

(20) Ellipticine (5,11-dimethyl-[6H]-pyrido[4,3b]carbazole) binds with an affinity greater than most other compounds known to interact with P-450. Control and 3-methylcholanthrene-induced aryl hydrocarbon (benzo[a]pyrene) hydroxylase (EC 1.14.14.2) and acetanilide 4-hydroxylase and control and phenobarbital-induced ethylmorphine N-demethylase "activities" are all markedly inhibited by ellipticine to about the same extent. Ellipticine and other "Type II" compounds (metyrapone, octylamine-1, pyridine, and aniline) preferentially inhibit NADPH-cytochrome P-450 reductase activity while affecting NADPH-cytochrome c reductase activity very little. Butanol-1, a compound having "pure Reverse Type I" character, does not block P-450 reductase activity like these Type II compounds. These data indicate that ellipticine may be very useful in attempting to understand the mechanism by which electrons are transferred from the reductases to the cytochrome(s) P-450.

(21) Whereas total P-450 content is more than four times greater in the rat adrenal cortex mitochondria than in microsomes, aryl hydrocarbon hydroxylase activity is more than 60 times higher in adrenal cortex microsomes than in mitochondria. The rat adrenal microsomal hydroxylase activity is strongly inhibited by α-naphthoflavone in vitro; progesterone 21-hydroxylase is not. In rats hypophysectomized for 30 days, aryl hydrocarbon hydroxylase decreases to about 6% of control values, but progesterone 21-hydroxylase falls to about 38% of that in sham-operated control animals. Hypophysectomy causes a striking decrease in an electrophoretic band estimated to be about 57,000 daltons. Aryl hydrocarbon hydroxylase specific activity and this electrophoretic band are restored to normal levels in adrenal microsomes of hypophysectomized rats that have received exogenous ACTH treatment. These data show that aryl hydrocarbon hydroxylase and progesterone 21-hydroxylase may be associated with different forms of adrenal microsomal P-450. The regulation of the adrenal hydroxylase by the large polypeptide hormone ACTH and the lack of inducibility by polycyclic aromatic compounds, however, are characteristics distinctly different from those of the P_1-450-associated hepatic enzyme.

(22) Membrane proteins which interact directly with the hydrophobic core of phospholipid bilayers have an absolute requirement for detergent to be maintained in the soluble state. Electrofocusing and electrophoresis of these proteins therefore pose special problems not encountered in similar work with soluble proteins. Nearly all studies of membrane proteins conducted thus far have utilized either Triton X-100 in combination with urea or sodium dodecylsulfate, both of which are strongly denaturing. The objective of our studies is, in contrast, the electrophoresis and electrofocusing of membrane proteins in which biological activity and/or native conformation are retained. The detergents which can successfully meet this objective must have several important properties. First, their solubilizing properties must be mild enough to produce non-denatured proteins, and yet strong enough to dissociate large aggregates. In addition, these detergents must have minimal effects on the surface net charge in order to give protein-detergent complexes which retain their native electrophoretic properties.

(23) In order to achieve the desired balance between solubilizing and denaturing properties of these detergents, as well as the maintainance of native charge, specific attention must be paid to both the "head" and "tail" groups of the detergent. In addition, the mode of association between monomeric or micellar detergent and protein must be considered.

(24) In an attempt to fractionate mouse liver cytochrome P-450 in its native state, electrofocusing systems were examined under conditions in which the surface net charge of solubilized proteins was preserved. A mixture of the zwitterionic detergent, SB_{14}, and the nonionic detergent, Triton X-100, appeared capable of completely solubilizing integral membrane proteins. Since charge properties were not altered, it was possible, for the first time, to focus basic membrane proteins in such detergent mixtures. The pH gradients (pI range 7-11) formed in the presence of these detergents were sufficiently stable to allow electrofocusing to the steady state of the solubilized membrane proteins. By the criterion of pattern constancy, these conditions were achieved within 15 hours, 0-4°C, at 200 V in 6-cm gels of 5% T/15% C_{Bis} with 0.1 N H_2SO_4 and 0.1 N KOH as anolyte and catholyte, respectively. It was expected that the native state of solubilized proteins could be maintained in such systems. Cytochrome P-450 proved to be denatured, however, by concentrations of these detergents required for complete solubilization of mouse liver endoplasmic reticulum.

Significance to Biomedical Research and the Program of the Institute: The cytochrome P-450-mediated monooxygenase systems metabolize steroids, cholesterol, fatty acids, indoles, sympathomimetic amines, and thyroxine, as well as lipophilic foreign substrates. Administration of polycyclic aromatic hydrocarbons to the pregnant animal induces this enzyme activity in the placenta and also transplacentally in several fetal tissues. Cigarette smoking is associated with increases in human placental aryl hydrocarbon hydroxylase activity. Ingestion of charcoal-broiled beef and many other foods by laboratory animals and humans is now known to be associated with increased intestinal metabolism of several drugs. We have demonstrated that allelic differences at a genetic locus controlling concentration of a receptor in the mouse is associated with: (1) increased susceptibility to 3-methylcholanthrene-initiated tumors; (2) decreased zoxazolamine paralysis time; (3) increased susceptibility to 7,12-dimethylbenz[a]anthracene-produced skin inflammation; (4) increased susceptibility to acetaminophen-induced hepatic necrosis and cataract formation; (5) shortened survival time, or (conversely) apparent protection, when large doses of polycyclic hydrocarbons, lindane, or polychlorinated biphenyls are administered intraperitoneally or orally; (6) apparent protection against aplastic anemia and leukemia caused by oral benzo[a]pyrene; (7) increased susceptibility to stillborns, fetal resorptions, and malformations caused by 3-methylcholanthrene or 7,12-dimethylbenz[a]anthracene given to the pregnant mother; and (8) increased ovarian toxicity (loss of primary oocytes) following polycyclic hydrocarbon treatment. An increased incidence of lung cancer in man has been correlated with living in highly polluted urban areas. It is therefore possible that differences--caused by a very small number of genes--in man are related to increased susceptibility to cancers, birth defects, tissue necrosis, drug metabolism, bone marrow disorders, and decreased life span caused by exposure to foreign compounds.

These genetically mediated dissimilarities in xenobiotic metabolism may be a useful probe in demonstrating differences in the rates of formation of certain drug-produced birth defects. Consequently, studies involving the development and extent of induction of mixed-function oxygenases in fetal tissues and in the placenta during various periods of gestation, are relevant to the program of this Institute in the following areas: (1) possible effects of environmental

pollutants (i.e., polycyclic hydrocarbons and insecticides) and various drugs on normal fetal growth and development and on the health of the pregnant woman, (2) mechanisms of "pharmacological immaturity" of the premature and newborn, (3) mechanisms of disease of the fetus and newborn concerning oxygenative and conjugative metabolism of normal body substrates such as steroids and bilirubin, and (4) possible therapeutic and diagnostic procedures for aiding and predicting pharmacogenetic defects in the fetus and neonate.

Proposed Course of Project: (1) Delineate further differences in the mechanism(s) of monooxygenase induction by polycyclic aromatic compounds, phenobarbital, and numerous other drugs and environmental pollutants, (2) characterize specific and nonspecific binding of these various inducers to subcellular sites, (3) examine the effects of hormones and nutrition on monooxygenase induction and associated intermediary molecules of importance such as cyclic-AMP, cyclic-GMP, and polyamines, (4) study various inhibitors and activators of monooxygenase activity in cell culture, (5) determine differences in metabolite formation, binding of reactive metabolic intermediates to DNA, and biophysical characteristics of the enzyme active-site between control and drug-treated animals or cells in culture, (6) compare these results in genetic differences with contemporary models of mammalian gene expression and regulation, and (7) search for drug metabolism parameters in cultured human lymphocytes or fibroblasts or in various excreta that might aid in predicting a patient's response to a drug or class of drugs; clinical studies attempting to demonstrate an association with the Ah locus for retinitis pigmentosa, idiopathic aplastic anemia, cyclic neutropenia, childhood leukemia, and pediatric solid tumors are now underway.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00137-05 DP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

GENETIC REGULATION OF DRUG-CONJUGATING ENZYMES

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	I. S. Owens	Research Biologist	DP	NICHD
OTHER:	N. Malik	Visiting Fellow	DP	NICHD
	C. Legraverand	Visiting Fellow	DP	NICHD

COOPERATING UNITS (if any)

D. W. Nebert and coworkers, Section on Pharmacogenetics & Molecular Teratology, DPB, NIH, NICHD
B. Burchell, Medical Sciences Inst., The University, Dundee, Scotland

LAB/BRANCH

Developmental Pharmacology Branch

SECTION

Unit on Drug-Conjugating Enzymology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
36/12	36/12	0/12

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Further studies concerning the detoxification of noxious, lipophilic foreign and endogenous compounds through oxidation by monooxygenase activity and/or through conjugation by UDP glucuronosyltransferase activity have continued primarily with the ubiquitous environmental pollutant, benzo[a]pyrene, and bilirubin. With biochemically produced 1-hydroxybenzo[a]pyrene as substrate for further metabolism *in vitro* studies with hepatic microsomes indicate that (1) certain monooxygenase activity further metabolizes the phenol to highly mutagenic products as well as generates a DNA binding profile very similar to that for the carcinogenic intermediates of benzo[a]pyrene and that (2) glucuronidation of the phenol by transferase protects against mutagenesis and partly against DNA binding. With bilirubin as substrate, characterization studies with immunoprecipitates of transferase by specific antibodies indicate that induced bilirubin transferase activity in hydrocarbon responsive C57BL/6N mice at 10 days after 3-methylcholanthrene treatment is highly labelled *in vivo* by pulses of radiolabelled amino acids, whereas transferase in the hydrocarbon nonresponsive DBA/2N mice is not significantly labelled under the same conditions. Further, characterization of other induced bilirubin transferases are under investigation.

Project Description:

Objectives: The removal of many foreign lipophilic compounds from the body requires both hydroxylation by monooxygenase activity and subsequent conjugation by sulfotransferase(s) or UDP glucuronosyltransferase(s). With inbred strains of mice and offspring from the appropriate backcrosses using two prototypic strains, research in this Branch has established that induction of several monooxygenase activities by aromatic hydrocarbon compounds is genetically regulated by a single gene designated the Ah locus. More recently, our studies have shown that induction of 4-methylumbelliferone-UDP glucuronosyltransferase activity is also genetically mediated and is linked to the Ah locus. The genetic linkage of these two functionally related membrane bound enzymes suggests that coordinate control might occur through the common intervening hydroxylated product of the monooxygenase activity which is also substrate for UDP glucuronosyltransferase activity. Since monooxygenases are important in the metabolism of drugs, carcinogens, and insecticides as well as endogenous compounds as fatty acids and cholesterol, it was of interest to investigate the extent to which monooxygenase is coordinated with UDP glucuronosyltransferase activity during the metabolism of a known potential carcinogen. Furthermore, carcinogenicity of benzo[a]pyrene, at least, appears to be associated with oxygenation whereas conjugation of oxygenated benzo[a]-pyrene appears to be detoxifying. In vivo data indicate that induction of the two enzymes systems are not necessarily coordinated through a metabolite of monooxygenase activity. The primary objectives of this project are (1) to delineate further the genetically mediated induction of UDP glucuronosyltransferase (using substrates which are products of the monooxygenase enzyme) in an attempt to determine substrate specificities of induced transferase(s) for various phenols of benzo[a]pyrene; (2) to explain further whether the parent polycyclic hydrocarbon or its oxygenated intermediate is the proximal inducer of the transferase; (3) to determine whether induced UDP glucuronosyltransferase, as assayed in vitro, reflects the increased in vivo conjugation of certain endogenous and exogenous substrates; (4) to exploit genetic differences in the conjugation of critical substrates, as bilirubin; (5) to study the functional interrelationship between UDP glucuronosyltransferase and various P-450-mediated monooxygenases in the detoxification of various drugs in vivo in genetically different mouse strains; (6) to use the bacterial mutagenesis system developed by Ames to examine the effect of UDP glucuronosyltransferase on the mutagenicity of certain compounds; (7) to attempt to use tissue culture techniques to study the regulation of mammalian UDP glucuronosyltransferase activity and the interdependence in regulation of the transferase and the monooxygenase at the molecular level; and (8) to apply tissue culture results and an in vitro bilirubin conjugation assay for the purposes of establishing a bioassay for "breast milk jaundice factor."

Methods Employed: The principal methods to be employed are: (1) enzyme assays using spectrophotometric, spectrophotofluorometric, radiometric determinations of product or disappearance of substrate, (2) in vivo radiometric measurements, (3) cell culture techniques, (4) differential centrifugation, (5) phase microscopy, (6) high-pressure liquid chromatography, (7) column chromatography, (8) variable temperature regulators, (9) gel electrophoresis, (10) antibody production in rabbit, and (11) immunoprecipitation of UDP glucuronosyltransferase.

Major Findings: Low concentrations of 1-hydroxybenzo[a]pyrene in the presence of NADPH and liver S-9 fraction from 3-methylcholanthrene-treated C57BL/6N mice is highly mutagenic, at least 3-fold more mutagenic than benzo[a]pyrene in the bacteria Salmonella typhimurium LT_2 tester strain TA98. With a similar system of liver microsomes from 3-methylcholanthrene-treated C57BL/6N mice and NADPH, [^{3}H]1-hydroxybenzo[a]pyrene is metabolized to reactive intermediates which covalently bind to deproteinized salmon sperm DNA in vitro. Enzymatically digested DNA, containing bound [^{3}H]1-hydroxybenzo[a]pyrene derivates, generates the same peaks of radioactivity as with [^{3}H]benzo[a]pyrene. All peaks resulting from [^{3}H]benzo[a]pyrene activation are represented by [^{3}H]1-hydroxybenzo[a]-pyrene. Similar DNA binding studies with microsomes from 3-methylcholanthrene-treated DBA/2N mice generate insignificant binding. Addition of substrate for glucuronidation of [^{3}H]1-hydroxybenzo[a]pyrene to the DNA binding assay in the presence of microsomes from 3-methylcholanthrene-treated C57BL/6N mice causes a reduction in radioactivity in certain peaks generated by [^{3}H]1-hydroxybenzo-[a]pyrene metabolism.

Studies with the endogenous substrate, bilirubin, show that induction of two peaks of bilirubin UDP glucuronosyltransferase activity (EC 2.4.1.17) with maxima at 6 and 10 days after a single treatment with polycyclic aromatic compounds, such as 3-methylcholanthrene and 2,3,7,8-tetrachlorodibenzo-p-dioxin, occurs in C57BL/6N, BALB/cJ and C3HeB/FeJ, but only the 6-day peak of activity occurs in DBA/2N, AKR/J and AU/SsJ mice. The induction of the activity which is maximal at 10 days follows the same pattern of inheritance as the induction of aryl hydrocarbon hydroxylase (EC 1.14.14.2) activity which is regulated by the Ah locus in these same mouse strains.

In treated offspring from the appropriate backcrosses and intercross between C57BL/6N and DBA/2N mice, the 10-day peak of induced activity is inherited as an additive trait while aryl hydrocarbon hydroxylase induction is inherited as a dominant trait. The more potent inducer, 2,3,7,8-tetrachlorodibenzo-p-dioxin, does not induce the 10-day peak of transferase activity in the nonresponsive DBA/2N mice, although this compound does fully induce aryl hydrocarbon in this mouse strain. Each mouse examined is responsive to the same extent for the 6-day peak of transferase activity. The declining activity 6 days after treatment in certain mouse strains is not due to loss of the transferase product, bilirubin monoglucuronide, by enhanced bilirubin monoglucuronide transglucuronidase activity in these mice.

Phenobarbital induces a similar profile of bilirubin UDP glucuronosyltransferase activity in both C57BL/6N and DBA/2N mice which is distinct from the activities induced by the polycyclic aromatic hydrocarbons.

The data indicate that (a) one form of inducible bilirubin UDP glucuronosyl-transferase activity and aryl hydrocarbon hydroxylase are coordinatedly regulated in these mice by the Ah locus and that (b) the mechanism of inducibility of this bilirubin transferase must necessarily encompass a restraint in DBA/2N mice which prevents full induction by the more potent inducer, 2,3,7,8-tetrachlorodibenzo-p-dioxin. These studies suggest that the existence of multiple inducible forms of bilirubin transferase in mice may have implications for humans with defective bilirubin transferase activity which is not responsive to barbiturate treatment. Bilirubin-UDP glucuronosyltransferase activity in

microsomes from either 6-day or 10-day 3-methylcholanthrene-treated C57BL/6N or DBA/2N mice or untreated mice was inhibited or activated to the same extent by 10 other substrates for glucuronidation. Five of these substrates as well as bilirubin are in a group considered neonatal and the other five are in a group considered late fetal. Compounds of either group did not prove consistent inhibitors or activators in native or detergent exposed microsomes. This evidence suggest that bilirubin transferase activities can not be precisely classified as either a neonatal or late fetal enzyme. Further characterization of the bilirubin transferase activity in the C57BL/6N and DBA/2N mice was continued by purifying control bilirubin-UDP glucuronosyltransferase activity to homogeneity and raising antibodies against the pure enzyme in rabbits. Transferase immunoprecipitates were formed from solubilized microsomal membranes, washed, and analyzed by SDS-polyacrylamide gel electrophoresis and fluorography. The bilirubin enzyme prelabelled in vivo by ^{35}S-methionine exposure and recovered from the immunoprecipitates showed that at 10 days after 3-methylcholanthrene treatment of the C57BL/6N mice bilirubin transferase was highly labelled, whereas the enzyme from similarly treated DBA/2N mice was very poorly labelled. Other aspects of the induction profile for bilirubin transferase in the two strains of mice will be analyzed by specific antibody studies.

Further studies with the genetic model of the aromatic hydrocarbon responsive mouse strain, C57BL/6N, and the hydrocarbon nonresponsive strain, DBA/2N, show that the UDP glucuronosyltransferase activities for the substrates, p-nitrophenol and 3-hydroxybenzo[a]pyrene, are induced in the C57BL/6N mice but not in the DBA/2N mice. The induction profiles appear to be maximal at 7-10 days after a single injection of 3-methylcholanthrene treatment. Transferase inducibility by 3-methylcholanthrene and phenobarbital in these mouse strains for the other eleven phenols of benzo[a]pyrene will be compared in an attempt to delineate the effect of the position of the phenol substitution in the benzo[a]pyrene molecule and efficacy of the compound as a substrate for transferase.

In Cell Culture UDP glucuronosyltransferase activity for 3-hydroxybenzo[a]pyrene appears to be induced about 2-3-folds in the Reuber hepatoma H-4-II-E established cell line by 3-methylcholanthrene, whereas transferase activity for p-nitrophenol is only slightly induced under the same conditions. Transferase inducibility for other benzo[a]pyrene phenols in this cell line will be compared as well as in mutant cell lines of H-4-II-E which have lost inducibility for monooxygenase activity.

Significance to Biomedical Research and the Program of the Institute: As discussed previously, drug oxygenations and subsequent glucuronide conjugations are catalyzed by a membrane-bound multistep enzyme complex. These enzymes are important in the process of detoxification and elimination of many normal body constituents and many xenobiotics taken in from our environment in the form of food additives, air pollutants, and medication. There are, as well, diseases of humans ascribed to defects (not well understood) in drug-conjugating enzymes. Examples in pediatrics include neonatal hyperbilirubinemia, Gilbert's syndrome, and the Crigler-Najjar syndrome. Therefore, the development of experimental model systems for understanding better the molecular regulation and neonatal development of UDP glucuronosyltransferase is pertinent to the program of the Institute in the following areas: (1) understanding the mechanisms of "immaturity of drug metabolism" in neonates; (2) providing further experimental

models for other human pharmacogenetic defects; (3) elucidating the basic enzymology of glucuronidating drug metabolism in order to gain insight into the development of selective inhibitors and stimulators of these drug conjugating enzymes that are useful for beneficial regulation of abnormal metabolism peculiar to various human diseases; and (4) understanding metabolism of various drugs in the fetus, premature and the newborn.

Proposed Course of Project: (1) Extend studies on activation of benzo[a]pyrene phenols to mutagens and reactive metabolites, (particularly the carcinogenic 2-hydroxybenzo[a]pyrene) and the effect substrates for conjugation has on activation of those phenols; (2) further exploration of the genetic difference in response to aromatic hydrocarbon treatment between certain inbred strains of mice to determine substrate preferences for the induced transferase; (3) examination of urine and bile for glucuronides of labelled exogenous substrates administered to treated responsive and nonresponsive mice; (4) determination of time course for glucuronide and other metabolites of the monooxygenase system, with a comparison of various carcinogenic and noncarcinogenic polycyclic hydrocarbons in the two genetic strains of mice; (5) further characterization of the transferase systems in cell culture with respect to number of different transferases, macromolecular syntheses requirements, and activators and general regulation at the molecular level; (6) determination of bilirubin tolerance in responsive and nonresponsive mouse after hydrocarbon treatment; (7) further examination of the effect of UDP glucuronosyltransferase on bacterial mutagenesis; (8) development of a useful bioassay in cell culture for the quantitation of "breast milk jaundice factor" in human breast milk samples from outpatients and/or inpatients; and (9) further purification of UDP glucuronosyltransferase and development of antibodies for enzyme characterization.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD-00500-01 DP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Hormonal Regulation of Gene Expression

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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LAB/BRANCH

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Unit on Hormonal Regulation of Gene Expression

INSTITUTE AND LOCATION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
42/12	42/12	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX(c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is concerned with biochemical and molecular aspects of hormone action during fetal development. Fetal liver is used as a cellular model for analyzing the mechanism of glucocorticoid hormone action. The glucocorticoid receptor is purified by DNA-affinity chromatography, and anti-receptor antibodies are prepared for use in determining the chemical structure and biochemical function of the receptor. The role of glucocorticoids in controlling the development of carbohydrate metabolism in fetal liver is studied *in utero* and *in vitro* using a chemically defined organ culture system. The fetal liver organ culture system is also used to study the synthesis of insulin-like growth factors (sommatomedins) at various stages of fetal development, and the synthesis of major secretory proteins (α-fetoprotein and albumin).

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: This project is concerned with biochemical and molecular aspects of hormone action during fetal development. The mammalian fetal liver is used as a cellular model for studying hormone action. Glucocorticoid hormones regulate many aspects of metabolism and gene expression in the fetal liver. A major goal is to understand the mechanism of action of glucocorticoids and to develop a comprehensive understanding of glucocorticoid effects on developing liver. The fetal liver is also a site of synthesis of insulin-like growth factors (sommatomedins) which mediate the action of growth hormone on linear skeletal growth. Biochemical characterization of these factors and study of their role in regulating fetal growth and metabolism are other major goals of the project.

Methods Employed: Fetal liver obtained from various mammalian species is maintained in organ culture in chemically defined medium. Hormonal effects on metabolism and enzyme induction can be studied without interference from serum components. Proteins synthesized and secreted by the liver are isolated and characterized by immunochemical and electrophoretic methods.

The cytosolic receptor for glucocorticoids is isolated by DNA-affinity chromatography. Anti-receptor antibodies are prepared and purified. Immunoaffinity chromatography is used to purify and characterize the glucocorticoid receptor.

Major Findings: (1) Development of antibodies to the rat liver glucocorticoid receptor. One of the major goals of the project has been accomplished during the past year. Antiserum that reacts with the rat liver glucocorticoid receptor has been produced using receptor purified by DNA-cellulose chromatography. This antiserum can be used to isolate the receptor from crude homogenates in a single chromatographic step. (2) Demonstration that fetal liver cultures synthesize a family of insulin-like growth factors (sommatomedins). Fetal rat liver in organ culture synthesizes small proteins (mw 10,000) which possess the chemical and biological properties of sommatomedins. These factors stimulate SO_4 incorporation into cartilage in vitro and stimulate DNA synthesis in cultured fibroblasts. They interact with specific sommatomedin receptors and also have weak insulin-like activity. The initial biochemical characterization of these factors has been completed, and their presence at high concentrations in fetal rat plasma has been documented. (3) Demonstration that glucocorticoids regulate the development of hepatic glucose-6-phosphatase. Previous work with fetal rat liver suggested that glucose synthesis and release from substrates such as galactose and fructose was regulated by glucocorticoids. Using a modification of the organ culture system which enables us to culture large quantities of fetal liver, we have shown that glucocorticoids increase the hydrolytic component of hepatic glucose-6-phosphatase activity during fetal development. (4) Demonstration of developmental difference in albumin processing. Albumin and α-fetoprotein are the major secretory proteins of the fetal mammalian liver. Both proteins are synthesized and secreted by fetal rat and mouse liver in organ culture. The ratio of α-fetroprotein/albumin synthesis is a useful biochemical marker for the developmental age of the liver. Developmental changes in this ratio take place in utero, but not in the defined culture system leading us to believe unknown exogenous factors

such as hormones or proteins may be required for developmental maturation of α-fetoprotein and albumin synthesis. Fetal mouse liver at early stages (gestational days 14-16) secretes two electrophoretically distinguishable forms of albumin. One of the forms has been identified as proalbumin; the other form is identical to adult albumin. Fetal rat liver cultured under identical conditions does not secrete proalbumin. This data suggests albumin processing is limited in the fetal mouse liver and this observation may be useful in identifying the developmental processes involved in albumin processing.

Significance to Biomedical Research and the Program of the Institute: The glucocorticoid receptor is extremely difficult to purify and characterize by standard biochemical techniques. The anti-receptor antibody provides a new biochemical probe for isolating the receptor and studying its function in regulating gene expression. Immunological approaches can be used for developmental studies and for analysis of receptor mutants.

Insulin-like growth factors are present in fetal plasma and amniotic fluid; however, their role in fetal growth and metabolism is unknown. The organ culture system has provided the first evidence that fetal liver is a site of sommatomedin synthesis; the culture system also provides an excellent, chemically defined system for studying the effects of growth hormone, insulin, and nutritional factors on the synthesis of these growth factors.

Proposed Course of Project: Application of the anti-receptor antibody to problems of glucocorticoid receptor structure and function will be a major focus of the project. The putative receptor protein can be isolated in a single step from crude preparations. Immunoreactivity is independent of steroid binding, and the activation state of the receptor. One of the first areas to be investigated will be the primary sequence of the receptor. Using mutant receptors with specified defects on glucocorticoid function and receptors exposed to limited proteolysis, we hope to define the functional domains of the receptor molecule. Covalent modification of the receptor by pyridoxal phosphate is thought to be important in blocking the DNA-binding function of the receptor. This hypothesis can be tested directly using the anti-receptor antibody. Important questions to be answered are whether multiple alleles for the glucocorticoid receptor are expressed in different tissues or at different times during development. We have already determined that the rat kidney glucocorticoid receptor does not react with the antibody, whereas the receptor from all other rat tissues does cross react. This data suggests that allelic variants of the receptor exist. Immunofluorescent studies are also feasible and may help to explain some of the genetic differences in the teratogenic effects of glucocorticoids. These effects (cleft face) occur at very early stages of development when biochemical studies on isolated fetal tissues are difficult.

Hormonal regulation of fetal sommatomedin synthesis will also be studied. We hope to determine if growth hormone regulates the synthesis of sommatomedins or sommatomedin binding protein in fetal liver. Precise immunoassays for fetal sommatomedins and sommatomedin binding protein are currently under development in laboratories of Nissley and Rechler.

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3. Eisen, H.J.: Significance of hepatocyte receptors. In Davidson, C.S. (Ed.): Problems in Liver Disease. New York, Stratton Intercontinental Medical Book Corporation, 1979, Chapter 8, pp. 86-90.

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5. Nissley, S.P., Rechler, M.M., Moses, A.C., Eisen, H.J., Higa, O.Z., Short, P.A., Fennoy, I., Bruni, C.B., and White, R.M.: Evidence that multiplication stimulating activity (MSA), purified from the BRL-3A rat liver cell line, is found in rat serum and fetal liver organ culture. Cold Spring Harbor, in press, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00501-02 DP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Non-Disturbing Investigation of Structure or Metabolism of Biological Systems Using ^{31}P Nuclear Magnetic Resonance Spectroscopy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

^{31}P is ubiquitous in biological systems, and the ^{31}P NMR technique enables signals from phosphates in tissues, cells, granules or macromolecules to be monitored directly. Since the ^{31}P NMR spectrum of ATP is sensitive to local pH, the internal pH of chromaffin granules was determined. To do this a calibration curve of ^{31}P chemical shift pH was obtained for the internal ATP for the first time. The results gave information on the function of the granule membrane and the process of exocytosis. Similar experiments are being carried out on intact cells also using internal P_i. The structure of DNA in the nucleosome core particle of chromatin is also being investigated by this technique; the results for natural and synthetic DNA's provide structural information of fundamental importance in understanding DNA function.

PHS-6040
(Rev. 10-76)

Objectives: To obtain reliable measurements of internal pH in cells and their components and to understand the processes of membrane transport of ions and protons, and the process of exocytosis. To compare phosphate metabolism in normal and diseased cells and to study the processes of phosphorylation of important macromolecules in situ. To study the structure of DNA and its interactions with proteins.

Methods Employed: High field phosphorus-31 nuclear magnetic resonance spectroscopy (^{31}P NMR).

Major Findings:

A. The Measurement of Internal pH by ^{31}P NMR: ^{31}P NMR is now widely used as a technique for the non-disturbing measurement of internal cell pH. The value of internal pH has been obtained by reference to a standard calibration curve for P_i or ATP-P_γ using a mixture of ionic components to approximate the cell interior. We have shown that a different calibration curve results for the titration of internal ATP-P_γ in chromaffin granules than is found for the mixture in free solution (Pollard et al., J. Biol. Chem. 254: 1170, 1979). This observation has now been extended to P_i titration curves. Contrary to explicit statements in the literature calcium phosphate (which is insoluble at high pH) and sodium phosphate do have different chemical shift and pK_a values. Also, two resolved ^{31}P signals were observed for P_i in the presence of DPL vesicles to which Ca^{2+} was added to the external solution. Although the differences observed are small they are of the same order of magnitude reported for the resolution of two P_i peaks observed in cells and muscles. The interpretation of such signals, in terms of specific pH compartments, by many studies in the literature may need re-evaluation as a consequence of these results.

B. Initial studies did indicate some differences in phosphate metabolism in mouse Le cells following treatment with interferon (ca. 10^{-9} M). Particularly levels of ATP were different between treated and control cells. However, significant variations were found from one preparation to another, which were traced to such factors as substituting phosphate buffer for sucrose as the final suspension medium. It has also been found necessary to block all ATPases in order to obtain reproducible results. This work is still in progress.

C. Structure of DNA in Nucleosome Core Particles: ^{31}P NMR parameters (δ, T_1, $W_{\frac{1}{2}}$ and NOE) were measured for the DNA in nucleosome core particles at three frequencies and compared with similar data for the histone-free DNA. An essentially linear relationship was found between frequency of observation and line width for the single broad envelope of ^{31}P resonances of the DNA in the nucleosome cores. We attributed this largely to chemical shift dispersion with smaller contributions from chemical shift anisotropy and dipolar broadening. These results suggest the presence of a range of different environments for phosphorus atoms in the core particles. Three other studies on nucleosome core particles, reported while this work was in progress, each were done at single observing frequencies, so that these conclusions are unique to our own work. Within the accuracy of the method, no asymmetry in the resonance could be detected, which would tend to rule out any significant degree of DNA

"kinking."

To investigate the interactions of the DNA and histones within the core particles we also studied transitions induced by urea and by temperature. Urea caused two step-wise increases in line width which we attributed to conformational changes. A biphasic transition was also observed in the temperature profile, consistent with previous optical studies (Weischet et al., Nucl. Acids Res. 5: 139, 1978).

Various models with different types of local mobility were examined by relaxation theory. A model of isotropic motion having a broad distribution of correlation times gave a fairly good fit to the ^{31}P NMR data. The local average phosphodiester mobility of 3×10^{-8} sec is six times shorter than that calculated on the basis of a rigid molecule.

D. Non-Uniform Backbone Conformation of DNA Indicated by ^{31}P NMR Chemical Shift Anisotropy: The ^{31}P NMR spectra of highly oriented DNA fibers (prepared by S. Zimmerman, LMB, NIAMDD) has been observed for both the A and B form conformations. The parallel orientation of the fiber axis with respect to the magnetic field exhibits a single, but abnormally broad, resonance. Theoretical considerations indicate that the ^{31}P chemical shift data for the B form fiber can be interpreted in terms of atomic coordinates proposed by Wilkins and that the phosphodiester coordinates must vary significantly along the DNA molecule. This latter conclusion is based in part on a computer simulation made by J. Wooten in which a theoretical spectrum was calculated which accounted for random fluctuations of DNA fibers from perfect alignment. Since even this fluctuation does not account for the broad resonance observed for B form DNA fibers, a more fundamental explanation is needed. This result and further considerations suggest that DNA has significant structural variations in its backbone conformation.

E. Structure of DNA: A highly homogeneous 145 base pair fragment of double helical poly(dAdT)·poly(dAdT) was obtained by micrococcal nuclease digestion of a semi-synthetic chromatin prepared from the nucleosome core histones and the synthetic polydeoxyribonucleotide. In contrast to higher molecular weight alternating copolymers, this fragment displayed two resolved ^{31}P NMR signals, separated by 24 Hz at 109.3 MHz. The two signals were of equal intensity at all temperatures less than the T_m for the fragment. Analyses of the possible origins for the two resonances leads to the conclusion that the phosphodiester backbone of this DNA contains two distinct phosphorus environments, probably in an alternating array. This may indicate the presence of sequence dependent local variation in the helical structure of DNA in general.

Significance to Biomedical Research and the Program of the Institute: (1) Prior attempts to measure internal cell pH have been difficult and intrusive. ^{31}P NMR has provided a nonintrusive method, but until now the quantitation of the method was incorrect. Now it should be possible not only to obtain accurate values of internal pH, but from the methods developed here, a greater understanding of ion transport through membranes and of the mechanism of exocytosis. (2) The presence of sequence-dependence local structural variations in DNA is of utmost significance, particularly for protein and drug recognition sites.

Proposed Course: (1) Further studies on chromaffin granules will be carried out utilizing the information we now have to study factors affecting changes in ΔpH and the process of exocytosis. (2) A calibration curve of internal P_i will be determined probably in mouse Le cells. (3) A treatment to block ATPases is being developed which should allow systematic studies of the effects of interferon on phosphate metabolism in mammalian cells. Attempts are being made to isolate and identify a major unknown metabolite observed in mouse Le cells. (4) Studies of 145 base pair poly(dAdT) in the presence of binding proteins will be carried out in an attempt to observe sequence specificity. A general method to prepare medium length oligomers is being developed to avoid the complex biochemical process used so far. This should greatly extend the range of these studies and allow us to test the generality of the structural conclusions. (5) Effects of drugs and mutagens on DNA in nucleosome core particles in solution will be monitored by ^{31}P NMR spectroscopy.

Publications:

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2. Granot, J., Elgavish, G., and Cohen, J.S.: Phosphorus-31 relaxation times of inorganic phosphate and nucleotides in aqueous solution. J. Mag. Res. 33: 569-575, 1979.

3. Shindo, H., Simpson, R.T., and Cohen, J.S.: An alternating conformation characterizes the phosphodiester backbone of poly(dAdT) in solution. J. Biol. Chem., in press, 1979.

NPMB, NICHD Annual Report October 1, 1978 through September 30, 1979

The Neonatal and Pediatric Medicine Branch has continued its broad research program in both clinical and basic approaches to the diagnosis, treatment and fundamental mechanisms of diseases of infants and children. Included in the program are genetic diseases, inborn errors of metabolism, disordered nutritional states, congenital malformations, endocrine and metabolic disturbances, as well as the effects of disorders of maternal origin on the neonate. This group also studies the normal biological mechanisms of infant and child development.

Currently, there are three Sections and 2 units actively carrying on these research projects. These include: 1) the Section on Developmental Biology and Clinical Nutrition with its units of Endocrinology and Neonatal and Pediatric Mineral Metabolism; 2) the Section on Human Biochemical and Developmental Genetics; and 3) the Section on Gastroenterology and Nutrition. An overview of each will be presented in brief.

I. The Section on Developmental Biology and Clinical Nutrition has ongoing investigations in three distinct areas: a) the developmental and hormonal mechanisms controlling phospholipid biosynthesis in various fetal and adult organs; b) determining the role of the central nervous system in the pathogenesis of massive obesity with or without the Prader-Willi syndrome, and c) the basic mechanisms involved in the diagnosis, treatment and pathogenesis of a number of metabolic disturbances including glycogen storage disease and hypoglycemia with or without hyperinsulinemia. The Endocrinology Unit,(A) is studying the mechanisms of early sexual development with a primary emphasis on improving methodology to measure Mullerian Inhibiting Substance (MIS). Other areas of study include the role of LH-RH in the development of the hypothalamic-pituitary-gonad axis. Collaborative studies have also been undertaken to explore the possibility of unusual thyroid hormone dysfunction in patients with cystinosis. The Neonatal and Pediatric Mineral Metabolism Unit (B) continues their research effort on metabolic and nutritional aspects of growth and development, mineral metabolism in infancy and childhood both in health and disease and endocrine functions occuring between conception and the attainment of adulthood.

a) The developmental studies of phospholipid biosynthesis have concentrated this past year on studying the effects of dexamethasone, thyroxine and hydrocortisone on choline kinase from rat liver cells grown in culture in collaboration with Dr. Paul Di Sant Agnese's laboratory and NIAMDD. The results of these studies indicate that ethanolamine kinase was stimulated at optimum concentrations of dexamethasone but choline kinase activity remained constant. Effect of insulin on choline kinase activity as well as the entire phospholipid biosynthetic pathway has been studied in lung organ cultures throughout the last trimester in the fetal rat. Preliminary studies suggest a striking effect of insulin on increasing choline kinase activity. Latter studies are being carried out in collaboration with Dr. Jesse Roth of NIAMDD.

b) The obesity studies have now been extended beyond the measurement of diurnal endocrine responses in the obese and weight reduced state to include investigations of basic central nervous system mechanisms involved in appetite and hunger. Attempts to correlate endorphin levels with specific dietary components, obesity and weight reduction have been initiated. Studies of bombesin and the neuro-endocrine pathways of hormone secretion are also being pursued.

The on-going investigation of glycogen storage disease, Type II, has progressed from the purification of human placental lysosomal (1-4) alpha-glucosidase to the production of antibodies to the renal and placental enzyme for urinary assays applicable to family studies. The purified enzyme will also be used to study the role of alpha-glucosidase in normal glycogen metabolism.

In addition, preliminary work has begun to develop techniques to study in vivo glycogen metabolism in obese and glycogen storage disease patients utilizing stable isotopes.

c) Methods to diagnose specific metabolic disorders such as type II glycogen storage disease, receptor defects in hypoglycemia and hyperglycemia and substrates such as ketone bodies, lactate, pyruvate and amino acids have been set up in order to better understand the pathophysiology of these disorders. Insulin receptor sites on red cells in a variety of disorders of carbohydrate metabolism have been measured throughout development and in patients with hypoglycemia both on and off diazoxide treatment.

All of these studies, are designed to determine and understand the basic mechanisms underlying normal growth and development. Animal models are being established to further investigate the mechanisms of the clinical oservations.

(A) The Endocrinology Unit, this current year, has produced the following results:

1) FSH inhibits MIS in rat testis.
2) Inactivation of LH-RH early in neonatal life permanently alters the male hypothalamic-pituitary axis.
3) The male hypothalamic-pituitary system is more sensitive than the female axis to inactivation of LH-RH with LH-RH antiserum. In addition, repeated injections in the female will delay puberty.

4) Patients with cystinosis consistently have abnormalities in hypothalamic-pituitary-thyroid function suggestive of partial thyroid hormone end organ resistance.

5) Prophylactic cranial radiation in patients with acute lymphocytic leukemia causes abnormalities in Gh secretion that correlate with abnormalities in the CT scan.

(B) In the Unit on Neonatal and Pediatric Mineral Metabolism, interests center on metabolic and nutritional aspects of growth and development, mineral metabolism in infancy and childhood (both in healthy children and during various disease states), and endocrine functions occuring between

conception and the attainment of adulthood. Having shown that endogenous fecal calcium loss is excessive in newborns by using stable calcium isotope modeling techniques, the effects of various hormones on endogenous fecal calcium and on calcium retention were investigated in the rat. Both calciferol and calcitonin were shown to increase calcium retention although only calciferol and parathyroid hormone decrease endogenous fecal loss. Attempts to measure levels of iron, zinc, and copper in extremely small samples revealed striking matrix interferance effects which can now be accounted for; accordingly, new tests for these essential trace elements in infancy were developed. Time series analysis of daily urinary gonadotropin excretion rates in a girl during the transition into the earliest stage of puberty disclosed a monthly cyclic pattern which changed in shape as pubescence began and a stable 13 day cyclic pattern not recognized previously in hormones of reproduction. Work will continue on characterizing the nutritional status of children with disorders effecting the assimilation and utilization of key dietary minerals (calcium, iron, zinc, copper, etc.), and urinary gonadotropin data from other girls and from boys will be analyzed for changes in cyclic patterns occuring during puberty.

II. The Section on Human Biochemical and Developmental Genetics continues both its basic science and clinical investigation of cystinosis and other defects in sulphur amino acid metabolism. Other current areas of research include amino acid transport, lysosomal structure and function, role of amino acids and fetal and neonatal nutrition and the biochemistry of early embryonic development. Continuing investigations of glutathione metabolism both in animals and in man have generated new approaches to the therapy of chronic hemolytic anemia in red cell enzyme deficiencies. Ongoing clinical investigations include the evaluation of cysteamine in reducing the storage of cystine in patients with cystinosis, and a collaborative world wide project to study the effectiveness of high doses of vitamin E (alpha tocopherol) in preventing the chronic hemolysis in glucose 6 phosphate dehydrogenase deficient patients. Both of these investigations are ongoing and definitive results will be forthcoming within the near future.

Major efforts continue on the incorporation of human genes into mammalian cells with the use of liposomes (lipochromosomes). Feasibility of producing lipochromosomes and using them to transfer X-linked genes has been previously reported. Work also continues on the development of a selective system for cystinotic cells in culture and demonstrating differences that exist between normal and cystinotic cells. The section actively pursues study of the effects of various drugs and biochemical pertubations in reducing the cystine content of cystinotic cells as well as studying detailed metabolic pathways and mechanisms in its pathophysiology.

A new contribution has been the demonstration that specific HLA types occur more frequently in patients with the recessive genetic disorder cystinosis than in a comparable normal population. These preliminary data represent the first demonstration of this relationship and will be extended to other genetic diseases as well.

In summary, studies will continue to focus in two areas. First the metabolism of amino acids and especially sulphur containing amino acids and peptides and inborn errors of metabolism involving these compounds. The second area will be on amino acids as they relate to nutritional

problems and intrauterine and post natal growth.

III. The Section on Gastroenterology and Nutrition continues the study of differentiation of the intestinal epithelial cells by using (a) cell and organ cultures and (b) a villus tip to crypt gradient of isolated epithelial cells. The activities of glycogen synthetase, phosphorylase and cyclic-AMP dependent protein kinase have been analyzed along the crypt/villus axis using isolated cells. Studies in progress include an analysis of cell maturation as determined by the activities of brush border membrane digestive enzymes (sucrase peptidase and alkaline phosphatase) following inhibition of cell proliferation. In conjunction with these studies the lab is also investigating the mechanism of cellular migration from crypt to villus tip and the subsequent exfoliation of the epithelial cell into the luman. At this time the lab is concentrating on three proteins (actin, myosin and fibronectin) which are thought to either propel the cells along the basement membrane or anchor them to it.

Current and planned studies include the initiation of a clinical service in gastroenterology with both an inpatient and oupatient component in order to attract patients for basic studies in the pathophysiology of both celiac syndrome and the disaccharidase deficiencies.

In conclusion, the clinical activities of the Neonatal and Pediatric Medicine Branch have expanded significantly over this current fiscal year. The establishment of a pediatric component to the weekly Inter-institute Endocrine Clinic has attracted patients from around the world. Research protocols have been generated based on these new opportunities. In addition, an Inter-institute Genetics Clinic spearheaded by this Branch has been initiated and is also beginning to generate patient-referrals and protocols. The short tenure due to fire of the expanded in-patient service of 23 beds was characterized by almost full occupancy at all times. Patients from NICHD accounted for 45 to 50 percent of the in-patient population. Although our clinical facilities are strictly limited at this time, the Clinical Program continues to thrive.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00126-05 NPMB

PERIOD COVERED

October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Developmental Biology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigator:

R.E. Ulane, Ph.D.	Chemist, DBCN Section	NPMB, NICHD

Other Investigators:

M. Cornblath, M.D.	Acting Chief, NPMB	NPMB, NICHD

COOPERATING UNITS (if any)

J. Roth, M.D.	Chief, DP	NIAMDD

LAB/BRANCH

Neonatal and Pediatric Medicine Branch

SECTION

Developmental Biology and Clinical Nutrition

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The control of fetal lung surfactant production is being studied by assessment of its principal phospholipid components, phosphatidylcholine (PC) and phosphatidylglycerol (PG). We have developed a fetal rat lung organ culture system capable of synthesizing PC in increasing amounts during a five day culture period, closely parallelling rates of PC biosynthesis in vivo during comparable gestational ages. Insulin was found to stimulate PC and PG biosynthesis and the specific activity of the enzyme choline kinase. Morphine sulfate was found to inhibit the biosynthesis of PC in fetal lung organ culture.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Studies on organ development have focused on fetal and neonatal lung as related to the respiratory distress syndrome (RDS) of human neonates. This disorder occurs in low birth-weight infants, is characterized by progressive pulmonary atelectasis, and accounts for greater mortality and morbidity than any other neonatal disorder or pediatric pulmonary disease. A decrease in the concentration of surfactant, composed primarily of disaturated phosphatidylcholine (PC) and a smaller but important amount of phosphatidylglycerol, (PG) are closely associated with the development of RDS. Thus, phosphatidylcholine and phosphatidylglycerol biosynthesis are essential to the development of a mature and functional pulmonary system. We have addressed ourselves, therefore to the following objectives: 1) To investigate the control of phosphatidylcholine and phosphatidylglycerol biosynthesis during fetal lung development. 2) To investigate the effects of diabetes, nutrition and drug addiction during pregnancy on phospholipid synthesis in fetal lung. 3) To establish a fetal lung organ culture system capable of undergoing differentiation *in vitro* to enable us to address the above objectives.

Methods Employed: PC biosynthesis is measured using isotopic substrates, extraction of labelled phospholipids, and separation into individual species utilizing thin layer and paper chromatography techniques. Fetal rat lung organ explants cultured in synthetic media are used as the model system. A rat liver cell line, capable of growing in the absence of serum, was also used to study the effects of various hormones on phospholipid biosynthesis.

Major Findings: An organ culture system has been developed which is capable of a normal developmental pattern of increasing rates of PC synthesis and increasing numbers of lameller bodies as a function of gestational age when incubated under appropriate conditions over a period of 5 days. Insulin was shown to increase the specific activity of choline kinase in these explants, while cortisol, dexamethotasone, and prolactin did not. Insulin was also shown to increase the amounts of choline incorporated into PrC as well as incorporation of palmitic acid into PC and PG. The insulin effect on choline kinase specific activity was studied in more detail in rat liver cells and was found to be due to synthesis of new enzyme rather than activation of existing enzyme. Morphine sulfate was found to inhibit in a dose dependent manner, the incorporation of choline into PC in lung explants.

Significance to Biomedical Research and the Program of the Institute: In order to study the metabolic disturbances leading to RDS, basic features of phospholipid metabolism in lung must be identified, particularly those factors which play a role in PC biosynthesis. We have taken the approach of looking into several disorders during pregnancy that many alter pulmonary maturation in the fetus. The observation that maternal diabetes is somehow involved in an increased incidence of RDS has led us to look into the relationship between insulin levels and the control of phospholipid synthesis.

Our studies have shown that increased levels of insulin stimulate PC synthesis in fetal lung, so that the increased incidence of RDS may not be a direct effect of fetal hyperinsulinemia as has been suggested. The reduced incidence of RDS in infants of mothers addicted to opiates promoted our investigations into the effects of morphine sulfate on PC biosynthesis. Our findings that morphine sulfate reduces the amount of PC synthesized in fetal lung explants is the first demonstration that opiate interaction can occur directly with the lung and manifest itself in a specific biochemical effect.

Proposed Course of Research:

The effects of insulin and morphine sulfate on fetal lung PC content will be extended beyond its initial biosynthesis to include its fatty acid content and subsequent secretion into the alveoli. Attempts will be made to demonstrate the existence of opiate receptors in fetal lungs. The mechanism of insulin induction of choline kinase will be investigated to determine whether this induction is mediated by specific insulin receptors. The effects of maternal diabetes and drug addiction on the fatty acid composition of PC and PG as well as the lamellar body content of the Type II cell of fetal lungs will also be studied both *in vivo* and in organ culture.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00127-05 NPMB

PERIOD COVERED

October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Developmental Endocrinology and Metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigator:

J.W. Hansen	Sr. Investigator	NPMB, NICHD

Other Investigators:

B. Corden	Clinical Associate	NPMB, NICHD
Nancy Vieira	Biologist	NPMB, NICHD

COOPERATING UNITS (if any)

H.J. Hoffman, Biometry Branch, NICHD
J.D. Schulman, NICHD

LAB/BRANCH

Neonatal and Pediatric Medicine Branch

SECTION

Section on Developmental Biology and Clinical Nutrition

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	10

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

XX

☐ (a1) MINORS ☐ (a2) INTERVIEWS

XX

SUMMARY OF WORK (200 words or less - underline keywords)

We are studying metabolic changes which occur as an individual develops from a fetus to a mature adult. We are currently focusing on two areas: 1) monthly gonadotropin cycles in premenarchial girls and pubescent boys utilizing radioimmunoassay methods for measuring urinary luteinizing hormone and urinary follicle stimulating hormone and analyzing the data with autoregressive computer techniques, and 2) mineral metabolism in the rat, monkey fetus, and human newborn. Calcium kinetics are studied in the patients using stable calcium isotopes analyzed in the laboratoy by mass spectrometry. Physiological effects of calcium are studied by measurements of ionized calcium in the blood. Trace element (zinc, copper and iron) analyses are performed by atomic absorption spectrophotomet as part of our studies of nutritional aspects of mineral metabolism in infancy and childhood.

Project Description:

Objectives: We are studying kinetics of calcium and trace element metabolism in the period around birth to understand better and to aid the newborn infant in its adjustment to extrauterine life. A special care nursery has been established to enable these and other clinical projects.

Methods Employed: Computer analysis of the data using autoregressive filtering techniques; urinary hydroxyproline and serum alkaline phosphatase, calcium, phosphorus, magnesium, zinc, copper, iron, total protein, albumin, pH, and ionized calcium measurements; calcium kinetic studies using stable isotopes and noninvasive urinary collection techniques for sample acquisition in newborn infants; mass spectroscopy of calcium isotopes; and radioisotope kinetic studies of calcium and trace element metabolism in laboratory animals.

Major Findings: Having shown that newborn calcium metabolism differs markedly from adult calcium metabolism, we used radiocalcium tracers to study possible influences of various hormones and nutrients on endogenous fecal excretion in the rat. This was possible only because endogenous fecal calcium excretion bears the same relationship to urinary excretion in the rat as it does in the newborn human. Vitamin D and calcitonin were found to improve calcium retention by increasing absorption, decreasing excretion and decreasing bone calcium resorption. Paradoxically, calcitonin increases endogenous fecal calcium loss. Medium chain triglycerides were also shown to increase fecal calcium loss and to decrease calcium retention when compared to a fat free diet, though this effect is probably less than a normal fat intake would produce.

Micromethods have been developed using graphite furnace atomic absorption to determine iron, zinc and copper. These techniques require less than 10 microliters of sample and thus make possible studies of trace elements in small newborns. Using these methods, normal adult zinc levels are 0.87 $\pm$ 26 ug/ml and copper are 0.99 $\pm$ 0.45 ug/ml. The iron technique has been used to begin work on developing a laboratory animal model for hemosiderosis and to find a good way to give iron by injection.

Using the statistical techniques of autoregressive filtering and frequency analysis on data collected for a period of one year from a prepubertal girl, we have confirmed the presence of monthly gonadotropin cycles and disclosed, for the first time, the presence of 13 day cycles.

Significance to Biomedical Research and the Program of the Institute: During the third trimester of intrauterine development, calcium and trace elements are accumulated in the rapidly growing fetus. With delivery, the newborn is removed from a rich supply of these nutrients via the placenta and is susceptible to deficiencies, hypocalcemia, and seizures. This is particularly true of the premature infant. The results of our studies should provide a rational basis for diagnosis and treatment of these infants with nutritional and metabolic problems relating to calcium

and trace elements. Studies on developmental endocrinology and metabolism in the period around birth have a direct bearing on the mission of the Neonatal and Pediatric Medicine Branch and of the NICHD and seek a better understanding of the process of normal growth and development and of the derangements accompanying various disorders.

Proposed Course of Project: Our research is progressively placing more emphasis on endocrinology and metabolism around the time of birth with initial studies being directed toward calcium control and metabolism in the fetus and newborn. We are also investigating trace element metabolism in the newborn as another aspect of developmental metabolism which requires elucidation to improve the nutrition of premature infants. The unique techniques we are using are also being extended to other children with disorders of mineral metabolism. This will give us a better understanding of disease processes and how we can more effectively treat them. We will also complete our analysis of samples previously collected to investigate gonadotropin excretion patterns during pubescence which is disclosing new information on the process of sexual maturation.

Publications:

1. Davis, S.E., Hansen, J.W., Bhatnagar, A.S. and Loriaux, D.L. Timed 12 hour specimens in outpatient estriol monitoring. J. Ob. Gyn., in press.

2. Chowdhry, P. and Hansen, J.W. Factors affecting calcium retention in the rat with implications for the newborn. Ped. Research 13:377, 1979

3. Hansen, J.W. and Hoffman, H.J. Urinary Gonadotropin cycles in prepuberta girls. Ped. Research 13: 379, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00131-05 NPMB

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Human Biochemical Genetics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigator:

J.D. Schulman	Head, Section HBDG	NPMB, NICHD

Other Invetigators:

E.J. Butler		
D. Towne		
K. MacDermott		
A. Mukherjee		
W. Nelson		
R. Steinherz		
V. Soutter		
J. Chemke	Visiting Scientist	NPMB, NICHD

COOPERATING UNITS (if any)

SEE ATTACHED

LAB/BRANCH
Neonatal and Pediatric Medicine Branch

SECTION
Human Biochemnical and Developmental Genetics

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
11	10	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS X ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our studies have concerned the metabolic errors of glutathione synthesis and degradation (glutathionuria, glutathione synthase deficiency and gamma glutamyl-cysteine synthase deficiency), glucose-6-phosphate dehydrogenase deficiency in which the capacity for glutathione reduction is decreased, cystinosis, homocystinuria, phenylketonuria, and non-ketotic hyperglycinemia. We have been interested in the development of newer forms of treatment and diagnosis for a number of these metabolic disorders and in understanding the mechanisms of disease production. A number of specific treatments are under investigation. Investigations have also been undertaken to explore the possibility of transfering human genetic material into mouse cells with the use of lipochromosomes. Some studies of the growth and metabolism of cultured amniotic fluid cells have also been undertaken in attempts to improve techniques for prenatal diagnosis.

PHS-6040
(Rev. 10-76)

COOPERATING UNITS:

L. Boxer, University of Indiana

J. Oliver, University of Connecticut

R. Berlin, University of Connecticut

M. Garrick, State University of New York

S.H. Mudd, NIMH

F. Tietze, NIAMD

A.D. Patrick, London (Inst. C.H.)

S. Kaufman, NIMH

S. Milstein, NIMH

C. Bartsocas, University of Athens, Greece

S.P. Spielberg, Johns Hopkins University

L. Corash, CC, NIH

F. Oski, Syracuse University

A. Eidelman, Jerusalem, Israel

J. Robbins, NIAMDD

V. Manganiello, NIHLB

K. Mittal, NBS

M. Sheetz, University of Connecticut

S. Moses, Beersheva, Israel

N. Bashan, Beersheva, Israel

B. White, NIAMDD

S. Wachtel, Sloan Kettering

J.L. Simpson, Northwestern

M. Rechler, NIAMDD

Project Description:

Objectives: The goals of the project are as follows: (1) to elucidate the basic biochemical defects responsible for selected inborn errors of metabolism in man; (2) to explore the secondary biochemical and physiological derangements caused by the primary metabolic defect; (3) to develop new or improved methods of treatment; (4) to develop new or improved methods of diagnosis, both antenatal and postnatal; (5) to use genetically abnormal cells and patients to expand understanding of normal human biochemistry, physiology, and development; (6) to see if human genes can be introduced into genetically deficient animal cells using lipid enclosed chromosomes. We have chosen to focus primarily on derangements of sulphur amino acid metabolism and secondarily on other disorders of amino acid metabolism and on selected derangements of lysosomal function. Our studies during this year have concerned the metabolic errors of glutathione synthesis and degradation (glutathionuria, glutathione synthase deficiency and gamma glutamyl-cysteine synthase deficiency), glucose-6-phosphate dehydrogenase (G6PD) deficiency cystinosis, homocystinuria, and phenylketonuria (PKU). Patients studied with abnormalities of glutathione synthesis include patients with severe acidosis (oxoprolinuria) and the adult, non-acidotic form of this disorder. Human genes transferred in lipochromosome experiments have included HGPRT, PGK and glucose-6-phosphate dehydrogenase.

Methods Employed: The principal methods employed are: (1) cell culture techniques, (2) spectrophotometric, radiochemical, and fluorometric enzyme assays, (3) differential ultracentrifugation (4) quantitative ion exchange chromatography of amino acids in physiological fluids, including cellular extracts, (5) radioisotopic measurements, (6) light, phase contrast, and electron microscopy, (7) column and thin-layer chromatography, (8) starch and polyacrylamide gel electrophoresis, (9) osmometry, (10) histochemistry, and (11) determinations of red cell and platelet survival after tagging with radioactive chromate.

Major Findings: Continuing investigations of glutathione metabolism in cultured human cells and in animals have permitted certain observations regarding the role of these enzymes and glutathione itself in biological processes.

Study of cells from patients deficient in gamma-glutamyl transpeptidase activity, as well as *in vivo* studies of one such patient, constitute the strongest evidence so far available that this enzyme is not, as previously believed, a significant mediator of amino acid uptake in a variety of human cell types.

We have shown that the adult form of glutathione synthetase deficiency without acidosis is due to a mutant glutathione synthetase which is unstable. Biochemical evidence for genetic heterogeneity among different patients with glutathione synthase deficiency and oxoprolinuria has been discovered. Successful correction of reduced intracellular glutathione content in the cells of patients with glutathione synthase deficiency has been achieved in vitro using a mixture of serine and borate, which inhibits gamma-glutamyl transpeptidase activity.

We have demonstrated that the cells from patients with reduced glutathione synthase activity are abnormally susceptible to oxidative damage as demonstrated by a shortening of red cell survival and a susceptibility to damage and microtubule dissociation in leukocytes during phagocytosis. Both the red cell and leukocyte abnormalities are partially and in some cases wholly correctable after the patients have received large doses of the antioxidant vitamin E for several months.

Furthermore related studies on the phenomenon of capping in leukocytes from patients with abnormalities of glutathione synthesis have helped to define the role of oxidized glutathione in microtubule assembly and disassembly.

Since glutathione may be involved in the deiodination of the thyroid hormones we are investigating whether cells from patients with glutathione synthase deficiency have abnormal metabolism of such compounds.

Glutathione is also considered necessary for the activity of GMP cyclase and investigations on the interrelationships between glutathione metabolism and GMP cyclase are currently underway using mutant and normal cells. In these investigations as in those involving thyroid hormone metabolism, glutathione depletion and oxidation using cystine starvation, diamide, and other techniques are also being utilized.

Since patients with glucose-6-phosphate dehydrogenase deficiency have a reduced capacity to regenerate reduced glutathione after oxidative stress, we have explored the possibility that the hemolytic anemia associated with this disorder might be improved by treatment with high doses of vitamin E. The rate of hemolysis in certain patients with chronic hemolyzing glucose-6-phosphate dehydrogenase deficiency is reduced with vitamin E treatment; this was also shown with the hemolytic anemia of glutathione synthase deficiency.

We have also demonstrated that the mild chronic hemolysis in Mediterranean type glucose-6-phosphate dehydrogenase deficiency is reduced by high dose vitamin E treatment in a population of Greek subjects. Significant increases in hematocrit and hemoglobin levels, reductions in reticulocyte count and significant prolongations of red cell survival were documented. It was observed that G6PD deficient individuals have significantly lower vitamin E levels than their controls. Further investigations in Greece and Israel will explore whether

Vitamin E treatment will reduce neonatal hyperbilirubinemia secondary to glucose-6-phosphate dehydrogenase deficiency or whether vitamin E administration will reduce the frequency and severity of the acute hemolytic crises responsible for the major morbidity and mortality in this common genetic disease.

Studies are underway exploring the susceptibility of glucose-6-phosphate-dehydrogenase deficient red cells to oxidative stresses in vitro (such as retinol-peroxide or aspirin-peroxide). The phospholipid membrane composition of G6PD and normal cells is also being compared under baseline conditions and during conditions of oxidative stress. The mechanisms of destruction of red cells in G6PD deficiency have also been examined with the use of lectins and an increased agglutinability by Concanavalin A of G6PD deficient red cells has been observed.

Alterations in the amount of the enzymes regulating the synthesis and degradation and glutathione have been demonstrated at different time points during fibroblast growth in vitro. These enzyme changes predictably alter intracellular glutathione content during cell growth.

Developmental changes in the amounts of the enzymes of glutathione synthesis and degradation and of intracellular glutathione content have been documented in the pre- and post-natal liver and kidney of the rat. The physiological significance of these alterations in intracellular glutathione content is being explored.

Investigations of cystinosis continue to be a primary interest in our laboratory. A number of significant observations on this disease have been made. A selective system has been developed for cystinotic cells in vitro based on the remarkable resistance of these cells to high concentrations of extracellular cysteine. We have demonstrated that ascorbic acid, which depletes intracellular cystine concentrations in vitro, does not constitute an effective therapy for the disease in vivo. However, current clinical trials with the use of cysteamine, cystamine and phosphocysteamine have demonstrated an in vivo reduction in leukocyte cystine content to near the normal range. Initial clinical observations on renal function are promising but not yet conclusive. Studies on the enzyme pantetheinase, a cysteamine generating enzyme, demonstrated its presence in human tissues for the first time and documented that pantetheinase deficiency is not responsible for cystinosis; in addition it has been demonstrated that a deficiency in endogenous cysteamine production cannot account for the increased intracellular storage of cystine in this disease. In additional studies on cystinotic and normal leukocytes, we have demonstrated that the glutathione metabolizing enzyme, gamma glutamyl transpeptidase is a natural component of lysosomes as well as the plasma membrane and the characteristics of this enzyme in cystinotic lysosomes are normal.

In additional studies we have observed that patients with cystinosis may suffer from frank or compensated hyhpothyroidism, and also that a regulatory defect involving the pituitary thyroid axis may exist in this disorder; increased resistance to thyroid hormone is suggested by the available data. We are presently examining in more detail the response of

the pituitary gland to graded doses of exogenous thyroid hormone in cystinosis.

Our interest in genetic diseases related to cystinosis has led to the description of a new genetic entity which resembles cystinosis clinically,

is more severe, but is not characterized by increased intracellular cystine storage; there is a unique combination of renal pathology and clinical symptomatology in this entity which has been described in siblings.

We are presently examining the permeability of isolated cystinotic and normal lysosomes to a variety of cystine-like compounds in efforts to establish whether the lysosomal storage of cystine is due to altered permeability characteristics of the lysosomal membrane. The synthesis of a number of novel disulfide compounds has been achieved in the course of these investigations and the capacity of these agents to induce cytoplasmic vacuolation in cystinotic and normal fibroblasts is also being explored.

We have uncovered some new aspects of the population genetics of cystinosis. The large excess of males over females observed in this disorder is not predicted by classical autosomal recessive inheritance. A striking linkage disequilibrium between cystinosis and the HLA antigen system has been demonstrated: HLA A3 and B7 are associated with cystinosis substantially more frequently than would occur by chance. We are currently exploring whether relationships to HLA markers can be used to provide mapping information with regard to the cystinosis gene.

Work is also continuing on non-sulphur amino acid inborn errors of metabolism. A new form of phenylketonuria due to decreased intracellular biopterin has been identified. A new in vivo assay for phenylalanine hydroxylase activity has been developed and tested. The assay involves the administration of minute amounts of tritiated phenylalanine to patients under an approved protocol. In one patient shown to have reduced phenylalanine hydroxylase activity on liver biopsy, the method was successful in revealing an equivalently reduced phenylalanine hydroxylase activity. This approach may lead to elimination of the need for phenylalanine loading tests and of liver biopsy in certain types of hyperphenylalaninemic patients.

We have concluded studies on exploring the effectiveness of the glycine antagonist strychnine in the treatment of non-ketotic hyperglycinemia. We have demonstrated that this agent improves muscle tone and feeding in affected infants but does not result in a substantial improvement in the long term neurological development. We have shown that the sodium valporate may be used in the symptomatic treatment of this disorder despite its tendency to produce hyperglycinemia under other circumstances. In laboratory animals we have completed studies on glycine entry into neonatal and adult rat neural tissue and have examined the effect of benzoate on enzymes which conjugate it with glycine, and on the clearance of glycine from various tissues including brain in the hyperglycinemic rat. These studies enhance our

understanding of possible disease mechanisms and approaches to treatment in this very serious disorder.

Our interest in other clinical genetic disorders has led to the description of a new disorder characterized by hyperuricemia and progressive pulmonary fibrosis. A new chromosomal rearrangement involving the X chromosome has been identified (pericentric X inversion). Furthermore, a unique individual with a 45, X karyotype and the presence of testicular tissue has been identified and studies on the relationship between his pattern of differentiation and the H-Y antigen are under investigation.

A number of studies are being undertaken in the areas of somatic cell genetics. We have demonstrated that human chromosomes can be enclosed in lipid envelopes and transferred into HGPRT deficient mouse A9 cells. This method of gene enclosure has resulted in a higher frequency of gene transfer and incorporation than previously reported. Large chromosomal fragments can be integrated into the recipient genome and such integration appears to have some site specificity. The suggestion that integration of chromosome fragments may occur at specific sites in the recipient genome is a novel observation with fundamental implications.

The mechanisms of meiosis have been of interest to some investigators in our laboratory. We are currently attempting to identify endonucleases which may be responsible for the single stranded DNA breaks thought to occur in meiosis. In other systems we have been exploring the effects of nutritional deprivation on patterns of meiosis and oocyte proliferation.

Because of the importance of prenatal diagnosis in many genetic disorders, we have been interested in improving techniques for a growing amniotic cell cultures. A controlled double blind study has now demonstrated that fibroblast growth factor from sheep brain can significantly stimulate the growth of human amniotic cell lines. Other studies involving prenatal diagnosis have included the demonstration that the prenatal diagnosis of the adrenogenital syndrome can be achieved by measuring 17 hydroxyprogesterone and androstenedione levels in amniotic fluid. With regard to other genetic disorders, the presence of insulin receptors is being sought in cultured human amniotic cells.

Significance to Biomedical Research and Programs of the Institute:
Cystinosis, homocystinuria, phenylketonuria, and the inborn errors of glutathione metabolism, all present clinical pictures of varying degrees of severity, in some cases lethal. These and other heritable disorders studied in our laboratory require better methods of diagnosis, treatment and prevention than are currently available, and our efforts in these directions are essential for the goals of this institute. In addition, it has been frequently demonstrated that the study of genetic variants in man is a powerful tool for advancing our understanding of normal and abnormal biochemistry. Our studies on glutathione metabolism illustrate

that point. Furthermore, the extension of our work from rare genetic disorders of glutathione metabolism to an extremely common one like glucose-6-phosphate dehydrogenase deficiency demonstrates how the study of rare model defects may open the way to understanding of common disorders with substantial public health significance. An interest in genetic diseases in general should include an interest in more fundamental aspects of human genetics related to mechanisms of cell growth, gene integration and other processes such as meiosis. Some of the studies cited above in our laboratories are consonant with this orientation.

Proposed Course of Project: Future efforts will include: 1) the continuing study of compounds which may be of potential therapeutic benefit in cystinosis and other heritable disorders; 2) further investigation of the mechanisms of lysosomal metabolism of cystine and related compounds in cystinosis; 3) continued investigation of the biochemical abnormalities in G6PD deficiency; 4) continued studies of the enzymes responsible for the synthesis and degradation of glutathione, and their involvement in disorders of its metabolism; 5) the development of an *in vivo* radiochemical assay for phenylalanine hydroxylase activity in patients with PKU and other abnormalities of phenylalanine metabolism; 6) investigation of additional inborn errors of metabolism; 7) continued studies of fundamental aspects of gene integration and the regulation of cell replication and meiosis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00132-03 NPMB

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Differentiation of Intestinal Epithelium

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigator:

N. Kretchmer	Head, DGN Section	NPMB, NICHD

Other Investigators:

S. Moses	Visiting Scientist	NPMB, NICHD
P. Burrill	Staff Fellow	NPMB, NICHD
K. Berry	Chemist	NPMB, NICHD
I. Bernardini	Chemist	NPMB, NICHD

COOPERATING UNITS (if any)
A. Guarino, NCI, Laboratory Toxicology
J. Bernstein, William Beaumont Hospital, Royal Oak, Michigan

LAB/BRANCH
Neonatal and Pediatric Medicine Branch

SECTION
Section on Developmental Gastroenterology and Nutrition

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.7	1.7	2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Section on Developmental Gastroenterology and Nutrition was established to study cellular differentiation and migration. The desirable model for this study is the intestinal epithelium because in a short period of time (48 hours), the undifferentiated crypt cell undergoes proliferation, migration, and differentiation. This orderly sequence of events in the jejunum of the rat provides a convenient investigative model. Differentiation gradients from crypt to villus are biochemically demonstrated by increasing levels of disaccharidases and ornithine carbamyl transferase. Thymidine kinase levels to evaluate cellular proliferation demonstrate increased activity at the undifferentiated cell level, but are not specific for intestinal cells. We are seeking by the application of biochemical, immunochemical and cytochemical techniques to define the role of actin, myosin and fibronectin in intestinal cell migration and adhesion. In addition we are continuing our efforts to understand the control of sucrose/isomaltase synthesis and the mode of incorporation of the complex into the intestinal brush-border-membrane.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Cell differentiation and migration are being investigated, using as a model the intestinal epithelium, where in a short period of time (48 hours) the undifferentiated crypt cell undergoes proliferation, migration, and differentiation. An orderly sequence of these events, progressing from the crypt to the villus, and established methods for the isolation of groups of cells at various levels of villus and crypt, makes this organ a convenient investigative tool. Our studies on cellular differentiation during the year have included (1) analysis of sucrase/isomaltase patterns in both cytosol and brush border fractions obtained from five regions of the villus and crypt of rabbit small intestine (2) characterization of the effects of a potent disaccharidase inhibitor on intestinal brush-border digestive and absorptive functions in the rat and on disaccharidase activity in sea lion (3) determination of glycogen phosphorylase and synthetase activities from crypt to villus in the rat and (4) the large scale preparation of sea lion brush-border-membranes for the purification of isomaltase. In addition, work in progress on cellular migration is focused on (1) the evaluation of the role of fibronectin in the attachment of epithelial cells to the basement membrane, (2) the clarification of the link between actin and myosin interactions and epithelial cell locomotion along the basement membrane, and (3) the effects of inhibitors of protein and DNA synthesis on cell migration and differentiation.

Methods Employed: The principal methods employed are: (1) cell and organ culture techniques (2) cryostat sectioning (3) cell dissociation techniques (4) animal surgery (5) light, phase contrast, immunofluorescent and electron microscopy (6) column chromatography (affinity, ion exchange, and gel filtration) (7) polyacrylamide slab and disc gel electrophoresis (8) centrifugation, ultracentrifugation and analytical ultracentrifugation (9) spectrophotometric, fluorometric and radiochemical assays (10) immuno-electrophoresis (11) autoradiography and (12) amino acid analysis.

Major Findings: Sucrase/isomaltase is an important enzyme complex that participates in the terminal (luminal) digestion of complex carbohydrates and dietary sucrose. This enzyme complex is located on the brush-border-membrane of the intestinal epithelial cell. The specific activity of this enzyme is low in the crypt region (the proliferative region of the gut), but shows a progressive increase concomitant with the maturation of other digestive and absorptive functions of the columnar cell. This laboratory found small molecular weight polypeptides which possess separate sucrase and isomaltase activities in the crypt and cytosol, where sucrase/isomaltase is not catalytically active *in vivo*. The identification of possible precursor peptides of the sucrase/isomaltase complex aids our effort to understand the control of the synthesis of this digestive enzyme complex, and the mode of incorporation of the complex into the brush-border-membrane.

During recent years several inhibitors of pancreatic alpha-amylase and intestinal alpha-glucosidases were purified from cultures of Actinomycetes, most frequently with strains of the genera Actinoplanes grown on specific carbohydrate-containing media, by the Bayer AG pharmaceutical company in Germany. A compound with the proprietary name of BAY G 5421 and with the structural features of an unsaturated cyclitol unit bound to 4,6 dideoxy-4-amino-D-glucopyranose was obtained from this company. The configuration of the cyclitol moeity is similar to that of intermediates formed during the course of the splitting of an alpha-glucosidic bond and thus may be a transition state inhibitor of alpha-glucosidases.

We have characterized the inhibitory activity of this substance on the following rat intestinal brush border membrane enzymes: sucrase, isomaltase, trehalase, maltase, lactase and glucoamylase. In addition, the effect of this substance on purified rabbit sucrase/isomaltase and sea lion brush-border membrane isomaltase activities was kinetically determined. The substance was a potent inhibitor of all enzymes tested, except lactase and trehalase, with Ki values ranging from 2.2×10^{-5} to 4×10^{-8} M. In addition, the transport of glucose from sucrose in vitro, using a tissue accumulation procedure, was retarded by this compound, whereas the transport of free glucose was unaffected. These results were confirmed in vivo by the demonstration of reduced blood glucose after a test load of sucrose plus inhibitor was given to rats. In a parallel study, a similar load of sucrose plus inhibitor increased respired H_2 in rats by a significant amount, when compared to controls without inhibitor.

Little is known about glycogen metabolism in the gut, or about its role in the proliferation and differentiation of the epithelial cell. The findings thus far indicate that glycogen phosphorylase activity is associated with crypt (proliferative) cells. These data are suggestive of a role for glycogen metabolism in cell division rather than in the digestive/absorptive functions of the intestine.

Cis-platinum is a potent inhibitor of cell division and is clinically used as an anti-tumor agent. This drug was found to markedly reduce cell proliferation in the gut and to subsequently reduce villus length. In addition, sucrase activity was significantly reduced following a single injection into rats (I.P.); whereas, the activity of another brush-border enzyme, alkaline phosphatase, was unaffected. Evidence of restoration of sucrase activity to normal was found on day five along with morphological data which indicated villus recovery.

Significance to Biomedical Research and the Program of the Institute: Definition of the processes involved in differentiation and cell migration may aid in the understanding of malabsorption states such as chronic diarrhea of infancy, short bowel syndrome, coeliac disease and other pathologic states where regeneration of the intestinal mnucosa is a major concern.

Proposed Course of the Project: The purification of actin and myosin and their respective antibodies is necessary in order to assess the relationship between these proteins and cellular migration. In addition mono-specific antibodies against fibronectin will aid us in our understanding of epithelial cell attachment to the basement membrane. The effects of agents (such as cytochalasin B, colchicine, actinomycin D, cycloheximide, etc.,) on cellular migration and actin, myosin and fibronectin synthesis and distribution in the epithelial cell will enhance our understanding of cell motility.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00133-02 NPMB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Study of Type II Glycogenosis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	J. B. Sidbury, M.D.	Scientific Director	NPMB, NICHD
	Lynn Davidson	Biologist	NPMB, NICHD

COOPERATING UNITS (if any)

LAB/BRANCH
Neonatal and Pediatric Medicine Branch

SECTION

INSTITUTE AND LOCATION
National Institute of Child Health and Human Development, Bldg. 10, Rm. 8D-45

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

There are now several forms of variants of myopathy associated with absent or deficient lysosomal α glucosidase. The classical Pome's disease, type II glycogenosis has an absence of the enzyme in all tissues except the kidney, and the heart is prominently involved. A juvenile form, i.e., later onset, presents simulating muscular dystrophy. Here, too, the enzyme is absent, but the heart is not clinically involved. This constellation can be seen in older children and adults where the myopathy is predominant. There is also a form of myopathy where the enzyme is markedly lowered and the Km 1/2 the normal. We are trying to isolate the enzyme from the urine to perform genetic studies and in order to use this source of snzyme to better characterize the enzyme.

Project Description

Objectives:
1. To study the properties of myopathies associated with increased glycogen and reduced lysosomal α glucosidase.
2. To further characterize the enzyme.
3. To produce antibodies to placental lysosomal glucosidase and renal cortical glucosidase.
4. To utilize the antibody to placental lysosomal glucosidase to study the role of lysosomal glucosidase in the normal metabolism of glycogen in fibroblast.
5. To develop antibodies to renal glucosidase to use in separating the renal from liver-muscle glucosidase.

Methods Employed:
Tissues are assayed for lysosomal α glucosidase using the umbilliferone pyranoside, following the fluorescence. The pH 4 and pH 6.5 activities are determined. The assay is run at different substrate levels and using different inhibitors and stimulators. The urine is assayed at pH 4 and pH 6.5. The urine is concentrated and run over a G-100 Sephadex column to separate the pH 4 and 6.5 activities. An improved method for separating these two activities is needed.

Major Findings:
We have found 2 patients with low, not absent, lysosomal α glucosidase and moderate glycogen accumulation. The patients presented clinically with myopathy. The enzyme was shown to have a V_{max} only 50% of that found in normal muscle. Clinically, there is the suggestion that the condition may be dominant, i.e., one mother-daughter involvement. Surprisingly, the glucosidase of the fibroblasts showed low activity but not an altered V_{max}. There are several possible interpretations of this.

The urine studies have been very frustrating. Contrary to claims by others, we find the enzyme activity in the urine to be variably stable to freezing. Unfortunately, that in the patients appears to be unstable, but we also have a number of normals whose urinary glucosidase is also unstable to freezing. We are so far unable to reproduce the Sephadex elution patterns for the urinary enzyme. We now feel that the unique glucosidase from the renal cortex makes interpretation of the urinary findings very difficult, if not impossible using current techniques.

Proposed Course:
We proposed to attempt to use antibody to placental glucosidase to study the role of the lysosomal glucosidase in normal cells. We will develop antibodies to renal glucosidase in order to be able to quantitate the renal vs. liver-muscle glucosidase in urine.

Publications:
None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00134-02 NPMB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Study of Obesity in Children with Prader-Willi Syndrome and Exogenous Origin

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.: J. B. Sidbury, M.D. Scientific Director NPMB, NICHD

COOPERATING UNITS (if any)
Clinical Center Nutrition Department and Physiotherapy Department
Nursing Department and Social Service

LAB/BRANCH
Neonatal and Pediatric Medicine Branch

SECTION

INSTITUTE AND LOCATION
National Institute of Child Health and Human Development, Bldg., 10, Rm. 8D-45

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Base-line glucose tolerance tests with insulin, prolactin release with thorazine, and the circadian rhythm of cortisol, GH, prolactin, LH, FSH are obtained. A low carbohydrate, low calories diet is initiated for weight reduction. Follow up studies of glucose tolerance and circadian patterns of the several hormones are repeated periodically during the course of weight reduction. The study will attempt to detect evidence of altered hormonal regulation in obesity with emphasis on the CNS role, including certain brain peptides.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

1. To determine insulin responses under different conditions in patients with Prader-Willi Snydrome (P.W.S.) as compared with exogenous obesity (EO).
2. To compare prolactin release after thorazine injection in the P.W.S. vs. EO and the circadian pattern.
3. To compare circadian pattern of hGH, LH, FSH, cortisol in P.W.S. vs. EO.
4. To determine and compare the levels of certain brain peptides (endorphins, bombesin, cholecystokin) to carbohydrate, protein or fat meals.
5. To determine the effect of weight loss on these parameters.

Methods Employed:

Patients with P.W.S. and EO who are at least 50% above their ideal weight for height are studied. Their "normal" caloric intake is estimated. A glucose tolerance test with determination of glucose and insulin is performed. The rise in prolactin following im injection of 0.7 mgm of thorazine/Kg ideal body weight is ascertained over 2 hours. The circadian pattern of LH, FSH, hGH, cortisol and prolactin is determined every 2 hours over a 24-hour period. The assays are performed by radioimmunoassay. The change in endorphins, bombesin and cholecystokin will be measured in the plasma following a carbohydrate, protein or fat meal. Comparisons will be made between the response in P.W.S., EO and normal weight individuals. The diet prescribed is low in carbohydrates and calories. Exercise is prescribed.

Major Findings:

The insulin response to glucose and a normal meal is exaggerated and prolonged. 80% of obese patients fail to show a prolactin release with thorazine administration. The very small number of patients whose circadian pattern for prolactin has been determined show a normal pattern despite the failure to respond to thorazine. The only patient who has failed to show a circadian rhythm for cortisol is a single post-pubescent P.W.S. No other post-pubescent P.W.S. patients have been studied. All prepubescent P.W.S. patients and EO patients have a normal pattern. The endorphin response has been uncertain for technical reasons. A clear-cut difference is found in the bombesin response between normal and obese patients following a carbohydrate meal. The response to the weight reduction program in both groups is similar.

Significance to Biomedical Research and the Program of the Institute:

Obesity is the major nutritional problem in the U.S. today. No abberations in metabolism or regulation have been discerned and confirmed except increased insulin levels, increased insulin resistance, and decreased insulin binding to cells and blunted hGH response to challenge. The present program is the beginning of a search for other abnormalities of regulation.

Proposed Course:
We plan to test the effect of Naloxone in appetite, behavior and the peptide response in the blood. Further studies will be done to elucidate the interaction of food substances with brain peptides and the sequential relationships of the peptides.

Publications:
None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00135-02 NPMB

PERIOD COVERED October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Developmental Endocrinology and Neuroendocrinology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigator: Barry B. Bercu

Other Investigators:
Merrily Poth, Clinical Associate, NPMB, NICHD
Gary Francis, Clinical Associate, NPMB, NICHD
Richard Haupt, Student Scientist
Abby Zlotnick, Student Scientist
Lisa Karam, Student Scientist

COOPERATING UNITS (if any)

SEE ATTACHED SHEET

LAB/BRANCH
Neonatal and Pediatric Medicine Branch

SECTION
Developmental Biology and Clinical Nutrition

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are studying the development of the hypothalamic-pituitary-gonad axis. We have developed a bovine sertoli cell culture system. We have raised antibodies to luteinizing hormone releasing hormone in rabbits. These antisera have been partially characterized as to specificity, antibody titer and biological blocking activity.

Patients in remission from acute lymphocytic leukemia and Ewings sarcoma had hypothalamic pituitary testing. Abnormalities in the regulation of GH secretion have been demonstrated in patients who received prophylatic cranial radiation and have leukoencephalopathy as diagnosed by computerized axial tomography scan. Our studies on cystinotic patients have revealed a consistent defect in the hypothalamic-pituitary-thyroid axis. We have also developed normal values of the pulse - wave arrival time (Qk_d interval) in children.

PHS-6040
(Rev. 10-76)

Cooperating units:

P. Donahoe, Massachusetts General Hospital

D. Poplack, A. Oliff, U. Bode, Pediatric Oncology, NCI

B. Schneider, Rockefeller Univ.

S. Reichlin and I.M.D. Jackson, Tufts - New England Medical Center

R. Johnsonbaugh, National Naval Medical Hospital

Y. Patel, McGill Univ.

J. Schulman, NICHD

D. Rodbard, NICHD

T. Triche, NCI

J. Butler, NICHD

A. Mukherjee, PRB, NICHD

Andrew Hoffman and Steven Paul, NIMH

Eugene Quinlan and Paul Kornblith,

Project Descriptions:

Objectives: The goals of the project are as follows: (1) to explore the development of the hypothalamic-pituitary-gonad axis; (2) to elucidate the biochemistry and function of two hormones produced by the sertoli cell, Mullerian Inhibiting Substance (MIS) and inhibin; (3) to explore intersex problems relating especially to MIS; (4) to elucidate hypothalamic pituitary control over the secretion of MIS: (5) to explore neuroendocrine regulation and dysfunction in a disease such as Kleine-Levin syndrome as well as in patients who have received prophylactic cranial radiation; (6) to develop new methods of measurement of these hormones. We have chosen to focus primarily on the bovine sertoli cell to study MIS and inhibin. Specific antibodies raised to luteinizing hormone releasing hormone (LHRH) are used to study the development of the hypothalamic-pituitary-gonad axis; (2) to elucidate the biochemistry and function of two hormones produced by the Sertoli cell, Mullerian Inhibiting Substance (MIS) and inhibin; (3) to explore intersex problems relating especially to MIS; (4) to elucidate hypothalamic pituitary and dysfunction in a disease such as Kleine-Levin syndrome as well as in patients who have received prophylactic cranial radiation; (6) to develop new methods of measurement of these hormones. We have chosen to focus primarily on the bovine Sertoli cell to study MIS and inhibin. Specific antibodies raised to luteinizing hormone releasing hormone (LHRH) are used to study the development of the hypothalamic-pituitary-gonad axis. We use a monkey model to evaluate the effects of cranial radiation on neuroendocrine function. In addition, patients with cystinosis have been studied to explore the possibility of unusual thyroid hormone dysfunction (partial end organ resistance).

Methods Employed: The principal methods employed are: 1) cell culture techniques, 2) radioimmunoassay of hormones, 3) hormone bioassays, 4) light, phase contrast, and electron microscopy, 5) partial purification of proteins and concentration techniques, 6) radioisotopic measurements, 7) raising of antibodies to hormones, 8) animal cannulation techniques, 9) measurement of cyclic AMP and cyclic AMP.

Major Findings: We are to develop a long term Sertoli cell culture technique. Partial characterization of the cells has been achieved with light and electron microscopy. Biochemical analysis of the cells is only partially complete.

Studies using low dose radiation to fetal rats suggest that the Sertoli cell produces MIS.

Antibodies to LHRH raised in rabbits and sheep are in high titer and do immunoneutralize LHRH. LHRH antiserum temporarily depresses serum gonadotropins in the rat and when injected in early life causes major changes in testicular development. The male hypothalamic-pituitary system is more sensitive to this pertubation than is the female system.

Using the immunoneutralization technique we have shown that LHRH controls MIS secretion. Further studies suggest the follicle stimulating hormone (FSH), not luteinizig hormone (LH), inhibits MIS secretion.

LH-RH injections cause uterine inertia in late pregnancy.

Abnormalities in growth hormone secretion occur in patients treated with prophylactic cranial radiation. In contrast, neuroendocrine regulation of other pituitary hormones appear to be normal. Further studies in humans and monkeys are continuing to further delineate these abnormalities.

Patients with cystinosis can have abnormalities in the hypothalamic-pituitary-thyroid axis. Studies are in progress to delineate whether the abnormalities are at the hypothalamic-pituitary and/or thyroid and/or peripheral tissue level.

Normals for pulse-wave arrival times or Qk_d intervals have been established in children.

In vitro studies of methylation reaction or pituitary cells in tissue culture are in progress. Correlation of endocrine function and methylation is being studied.

We are studying the effects of GH and somatomedin on craniopharyngioma in tissue culture. We are trying to relate this to clinical treatment of children with craniopharyngiomata.

We are studying whether there is a sex predeliction we are studying the implantation position of males and females in rats.

Significance to Biomedical Research and Programs of the Institute; The focus of our laboratory is to develop better methods of diagnosis and treatment of disorders of developmental endocrinology and neuroendocrinology. This includes intersex problems, especially those that require the correct diagnosis and treatment in the newborn period. In addition, we have studied developmental neuroendocrine complications of cranial radiation, a commonly used technique in pediatric oncology. Our efforts in these directions are essential for the goals of this institute because of their applications for advancing our understanding of child development.

Proposed Course of Project: Future efforts will include: 1) the continuing study of the development of the hypothalamic-pituitary-gonad axis; 2) further investigation of Sertoli cells and their functions; 3) continued studies of the abnormalities of the hypothalamic-pituitary axis of humans and monkeys MIS secretion; 5) further chemical characterization of MIS and inhibin; 6) investigation of intersex problems and Kleine-levin syndrome; 7) continued studies of the hypothalamic-pituitary-thyroid and gonadal axis in cystinosis.

Publications:

1. Bercu, B.B., Y. Morikawa, I.M.D. Jackson, and P.K. Donahoe. Inhibition of Mullerian Inhibiting Substance Secretion by FSH. (Pediatric Research.

2. Beitins, I.Z., M.L. Dufau, K. O'Loughlin, B.B. Bercu, J.W. McArthur, J.D. Crawford and K.J. Catt. Comparison of biological and immunological activities of serum LH in normal women during LHRH infusion and patients with Turner's Syndrome treated with estrogens. (In press).

3. Bercu, .B., I.M.D. Jackson and S. Reichlin. Delayed puberty after immunological blockade of endogenous LHRH in female rats. Proc. Soc. Biol. Med. 160:123-125, 1979.

4. Bercu, B.B. and P.K. Donahoe. Mullerian inhibiting substance in abnormalities of sexual differentiation, OB GYN Series (in press).

5. Bercu, B.B. and j.D. Schulman. Genetics of abnormalities of sexual differentiation and of female reproductive failure. OB Gyn Survey (in press).

6. Bercu, B.B., Donahoe, P.K.D., and J.D. Schulman. Developmental abnormalities of the Mullerian duct. OB GYN Series (in press).

7. Oliff, A., Bode, U., Bercu, B.B., DiChiro, G., Graves, V. and Poplack, D.G. Hypothalamic-pituitary dysfunction following CNS prophylaxis in acute lymphocytic leukemia: correlation with CT scan abnormalities.

8. Bercu, B.B., Orloff, S., and Schulman, J.D. Partial target organ resistance to thyroid hormone in nephropathic cystinosis. Society for Pediatric Research, Atlantic, May, 1979.

9. Oliff, A., Bode, U., Bercu, B.B., DiChiro, G., Graves, V., Pizzo, P., Glaubiger, D., Glatstein, E., and Poplack, D. Abnormally low growth hormone (GH) responses to insulin induced hypoglycemia following central nervous system (CNS) Prophylaxis: correlation with abnormal computeried tomographic (CT) brain scans. ASCO, Spring, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00401-01 NPMB

PERIOD COVERED

October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Disorders of Carbohydrate Metabolism in Infancy and Childhood

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigators: Marvin Cornblath

Other Investigators: George Chrousos
William Nelson III
Anthony Adams

COOPERATING UNITS (if any)

Jesse Roth and Helena Wachslicht-Rodbard, Diabetes Branch, NIAMDD

LAB/BRANCH

Neonatal and Pediatric Medicine Branch

SECTION

Developmental Biology and Clinical Nutrition

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

X

☐ (a1) MINORS ☐ (a2) INTERVIEWS

X

SUMMARY OF WORK (200 words or less - underline keywords)

Our major goal is the elucidation of the pathophysiology in infants and children with disorders of carbohydrate metabolism. This should provide a better understanding of the physiology of carbohydrate regulation as well as the developmental role of hormones and their receptors. Diet and other therapeutic modalities will be evaluated not only in treatment but also on the neurological and psychological development of the young patients.

Our studies of Nesidioblastosis, Leucine Sensitivity, the Beckwith-Wiedemann Syndrome and Glycogen Storage Disease Type I have shown: (1) some of these patients have increased insulin receptors on their erythrocytes, which might explain their symptomatic hypoglycemia as an increased response of peripheral tissues to insulin. (2), insulin receptor values on erythrocytes Since newborns have increased insulin binding, these data indicate a decrease in the numbers of receptors early in extrauterine life. In preliminary studies, Diazoxide decreased the insulin receptors.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

The goals of the project are as follows: (1) to elucidate the pathophysiologic mechanisms involved in various disorders of carbohydrate metabolism in infants and children. (2) To evaluate the currently used diet and therapeutic regimens in the above disorders and modify them as indicated. (3) To study the relationship of hormone effectors, their receptor sites and other parameters relevant to carbohydrate metabolism such as concentrations in blood of glucose, lactate, pyruvate, free fatty acids, ketones, and amino acids. (4) To assess the overall growth and development of these patients by following their growth patterns, the maturation, function and response of their neuroendocrine and peripheral endocrine systems as well as the development of their neurological and psychological behavior.

Methods Employed:

The research methodology employed can be separated in (a) clinical and (b) laboratory.

(a) The patients are studied clinically on the 9W pediatric ward of NICHD. Complete physical evaluations, neurological examinations and psychological testings are performed. Diagnostic tests available routinely at the Clinical Center will include EEG, diagnostic x-rays, CAT scans, clinical pathology tests etc as indicated.

(b) Laboratory investigative tests are performed to study three distinct, but related areas: (1) Insulin receptor sites (number of receptors and affinity to insulin) on circulating erythrocytes, measured by a radioreceptor assay, (2) Circulating insulin, growth hormone cortisol, T_4, and glucagon measured by radioimmunoassay and (3) biochemical determination of substrates and metabolites that either reflect or influence carbohydrate metabolism by micro-automated fluorometric techniques. Liver enzyme studies will also be done as indicated.

Major Findings:

(1) Normal values for erythrocyte insulin receptors in infants and children were established and found to be identical to those in the adult. Since the erythrocytes of neonates show increased insulin binding at birth and infants as young as 6 months old were found to have adult levels of insulin receptors, it would appear that some developmental changes in early extrauterine life occur that is expressed by a regression of insulin binding. This decrease does not correlate with any known changes in plasma insulin or growth hormone levels, although growth hormone values fall after the first weeks of life.

(2) Increased specific binding of insulin to erythrocytes was found in patients with hyperinsulinism syndromes that might be expected to have decreased receptors (down-regulation). Thus, our groups of patients were shown to have failure of down-regulation. Previously such failure has been shown only in infants of diabetic mothers.

(3) The greatest increase in the specific binding of insulin to erythrocytes was found in those patients who had persistent symptomatic hypoglycemia. Since it has been demonstrated that tissue response to insulin increases with increasing insulin binding we concluded that in these patients, the increased insulin binding might increase peripheral tissue glucose uptake in response to insulin and partly account for the symptomatology observed.

(4) We could not explain why our older infant patients with hypoglycemia had increased binding, similar to that of newborns. We postulated that this might constitute a persistence of the fetal state due to a derangement of factors that regulate the ontogeny of insulin receptors.

(5) A discrepancy was found between a child with Glycogen Storage Disease type I who was receiving continuous nocturnal feedings with Vivonex (high binding) and another with the same disease but receiving oral food (normal binding). We suggested that this may have been due to the stimulation of insulin secretion by the gastric inhibitory polypeptide which was stimulated by the continuous feeding. These observations require follow up and expansion to more patients with GSD Type I.

(6) In two patients the chronic use of Diazoxide^{R} seemed to cause a decrease in the number of receptors. This finding also needs verification because Dizoxide^{R} is a drug used widely in infancy and childhood hypoglycemia and in hyperinsulinism states.

(7) A family with two children with documented nesidioblastosis was studied. Abnormal glucose tolerance tests done at the 35th and 38th week of her gestation with the second child were found to be abnormal with marked reactive hypoglycemia in the mother. A glucose tolerance test done when the mother was not pregnant was normal. The first child had elevated insulin receptors on her erythrocytes, the mother during pregnancy had normal and the cord blood of the second child had normal insulin receptors in the presence of extremely high insulin levels (75 uU/ml).

Significance to Biomedical Research and Programs of the Institute:

The focus of our research is to elucidate the pathophysiology of disorders of carbohydrate metabolism in infancy and childhood and develop better methods of diagnosis and therapy. Through these efforts the development of the normal child will be better understood. Both objectives are in concert with the goals of the Institute.

Proposed Course of Project:

Future efforts will include: (1) the continuing study of the pathophysiology of carbohydrate metabolism disorders in infancy and childhood. (2) a detailed study of the ontogeny of insulin receptors and the interrelationships of insulin and insulin receptors, throughout early development. (3) evaluation of the efficacy and effects of continuous nocturnal nasogastric feedings in children with Glycogen Storage Disease type I and its effects on the carbohydrate regulatory system. (4) The effects of Diazoxide^{R} in the treatment of hypoglycemic syndromes in infants and children and elucidation of its mechanism of action especially concerning receptor and postreceptor events. (5) Further study of nesidioblastosis which seems to be a familial disease. (6) The role and interrelationship of glucagon, somatostatin, insulin and growth hormone with insulin receptors and the overall change during growth and development.

Publications:

1. Chrousos, G.P., Wachricht-Rodbard, H., Nelson, W. III., Roth, J. and M. Cornblath; Insulin binding to erythrocytes in infants with abnormlaities of glucose metabolism. J. Clin. Endocrinol. Metab., Submitted for publication 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00402-01 NPMB

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Mass Spectrometry Facility

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Alfred L. Yergey	Chemist	NPMB, NICHD
OTHER:	Edward Seeliger	Clinical Associate	NPMB, NICHD
	Lawrence Tamarkin	Staff Fellow	NPMB. NICHD

COOPERATING UNITS (if any)

J. Hansen, NPMB, NICHD

LAB/BRANCH

Neonatal and Pediatric Medicine Branch

SECTION

INSTITUTE AND LOCATION
National Institute of Child Health and Human Development, Bldg. 6, Rm. 140

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Solids inlet probes are being used for quantitative analysis in three areas. (1) Measurement of calcium stable isotope ratio using a thermal ionization technique. Tracer studies of calcium metabolism in animals and neonates are being carried out. (2) Analysis of amino acids using isobutane chemical ionization. Tracer studies using ^{13}C alanine isotope ratios in anaimal models are being undertaken. (3) Slow pyrolysis techniques are being developed for analysis of peptide hormones. Initial quantitative studies with this technique are being carried out using acetate derivatives of phospholipids.

PHS-6040
(Rev. 10-76)

Project description:

Objectives: The objective of this facility is to provide mass spectrometric analyses of materials encountered in programs of the institute.

Methods Employed: The emphasis of this facility is a quantitative analysis by use of the solids probe inlet, although the capability exists for GC sample processing and for qualitative or identification studies.

Major Findings: During the past year the facility's three quadrupole mass spectrometers have been made fully operational. Two of these instruments, Scientific Research Instruments BIOSPECTs, are devoted exclusively to chemical ionization (CI) applications and, at the present, are used in solids probe inlet mode only. The third instrument, a Finnegan 4021, is used extensively in the electron input (EI) mode, and is the only quadrupole in existance used for thermal ionization studies. It is capable of CI operation, and has a GC inlet. Data processing capabilities include an INCOS GC/MS data system that can be interfaced to any of the three instruments and the NIH Isotope Ratio Calculator, a micro-processor based specific ion monitoring device, that was developed during this year for quantitation studies.

There are three areas of quantitative mass spectrometry that are currently active.

(1) A method for measuring isotope ratios of calcium and other IA and IIA elements using thermal ionization at the solids probe tip has been developed. Natural abundance ratios have been measured for 4 of the 6 calcium isotopes with accuracies of about 1% while maintaining a coefficient of variation of about 1%. Isotope dilution studies using ^{44}Ca have shown that enrichments as low as 7% of natural abundance can be measured with a coefficient of variation of about 1%. Stable isotope tracer studies of calcium metabolism in animals and neonates are being carried out with NPM, NICHD.

(2) An analysis for amino acids using isobutane chemical ionization is being developed. Linear responses for various dilution curves in the range of plasma levels of free amino acids have been obtained to date. The purpose of this study is to develop tracer studies using ^{13}C-labeled amino acids as a probe into carbohydrate metabolism in glycogen storage diseases and as a means of studying neonatal nutritional needs.

(3) Analysis of peptide hormones is being undertaken by using slow pyrolysis of samples directly inside a chemical ionization source. The temperature dependent mass spectra represent sequential thermal degradations in the sample. Differentiation of materials is done according to variations in the shapes of the specific ion profiles of particular masses. The method has been shown to be very effective in distinguishing closely related cellular materials, and has excellent possibilities for differentiating peptide hormones from each other. This may permit quantitation of these materials. Calibration

of the apparatus is being carried out by using acetate derivatives of disaturated phospholipids in ammonia chemical ionization. Linear response curves have been obtained for mixtures of two such compounds over a 100-fold range.

Proposed Course: We plan to continue these specific projects as outlined and to provide analyses of particular samples for various investigators as they arise from time to time.

Publications: None

1979 ANNUAL REPORT
Pregnancy Research Branch

Project Numbers	Project Title	Principal Investigator
Z01 HD 00026-04 PR	Fertilization and Activation of Development in Mammals	B. Gulyas
Z01 HD 00029-05 PR	In Vitro Studies on Primate Luteal Cells	B. Gulyas
Z01 HD 00030-06 PR	Characterization of Patterns of Chorionic Gonadotropin Production in Primates	G. Hodgen
Z01 HD 00031-06 PR	Hormonal Interrelationships Between Fetus, Mother and Placenta in Primates	G. Hodgen
Z01 HD 00045-04 PR	Regulation of Folliculogenesis in the Monkey	G. Hodgen
Z01 HD 00168-03 PR	Ovarian Xenobiotic Metabolism and Oocyte Toxicity	D. Mattison
Z01 HD 00177-02 PR	The Cynomolgus Monkey (Macaca fascicularis) as an Appropriate Surrogate	G. Hodgen
Z01 HD 00900-01 PR	Factors Regulating Estrogen and Progesterone Receptors in Monkey Endometrium in the Fertile Menstrual Cycle	G. Hodgen
Z01 HD 00901-01 PR	The Core Endocrine Laboratory Unit	W. Nixon
Z01 HD 00902-01 PR	Developmental Enzymology	J. Chou
Z01 HD 00903-01 PR	Enzyme induction in a genetically deficient animal by normal hepatic tissey transplantation	A. Mukherjee
Z01 HD 00904-01 PR	Chromosome mediated gene transfer in mammalian cells	A. Mukherjee
Z01 HD 00905-01 PR	Development of in vitro selective system for cystinotic cells	A. Mukherjee

ANNUAL REPORT
of the
Pregnancy Research Branch
National Institute of Child Health and Human Development
October 1, 1978 through September 30, 1979

SUMMARY

The current research program of the Pregnancy Research Branch focuses upon the physiology of gestation (including genetic and developmental factors which contribute to perinatal fetal and maternal morbidity and mortality), fertility regulation, toxicological aspects of ovarian function, as well as fertilization and implantation. Both basic and clinical aspects of the reproductive process are being investigated. Besides these research activities, the Branch trains Associates and Visiting Fellows in laboratory and clinical investigation by participation in the combined program in Reproductive Endocrinology that has been established with the Armed Forces Institute, specifically for career development in obstetrics and gynecology. Also, the combined program with NIAMDD is available to Clinical Associates interested in Endocrinology.

The rodent ovary contains the microsomal monooxygenases capable of metabolizing polycyclic aromatic hydrocarbons to reactive intermediates. The polycyclic aromatic hydrocarbons; benzy(a)pyrene, 3-methylcholanthrene and 7,12-dimethylbenz(a)anthracene destroy primordial oocytes in the rodent ovary in a time, dose and metabolism dependent manner. The goal of this research is to explore the relationship between ovarian metabolism of xenobiotic compounds and effects on reproductive performance. Using inbred strains of mice and rats, we have investigated the time, dose and metabolism dependence of oocyte and follicle destruction. Initial studies utilizing inbred DBA/2N (D2) and C57BL/6N (B6) mice demonstrated differences in response of the ovarian microsomal P-450 dependent monooxygenases to PAH tratment, D2 mice treated with BP had no change in ovarian aryl hydrocarbon hydroxylase (AHH, benzo(a)pyrene 3 hydroxylase, EC 1.14.14.2) activity, but B6 mice treated with BP had a three fold increase in ovarian AHH activity. After treatment with the PAH, BP, MC, and DMBA, primordial ooxcytes were destroyed in both strains of mice in a time and dose dependent fashion. Oocyte destruction with all three compounds was more rapid in B6 than D2 mice and the ovotoxicity of the three PAH was BP < MC < DMBA. The competative inhibitor of PAH metabolism, α-naphthoflavone blocks oocyte toxicity by the PAH, and the nonmutagenic PAH pyrene is not ovotoxic. These data suggest that oocyte destruction is specific and dependent on metabolism. The specificity also extends to cell type. BP and MC seem to affect only the primordial oocytes with no demonstrable light or electron micrographic changes in the surrounding granulose or ovarian stromal cells. DMBA, however, is much more toxic and destroys primordial oocytes and primary follicles as well as growing and large follicles. Comparing ovarian AHH activity and oocyte toxicity in D2 and B6 mice suggested an association between inducible ovarian AHH activity and oocyte toxicity. To further explore this relationship, we have investigated PAH oocyte toxicity as well as the metabolic profile of BP in Sprague-Dawley (SD) rats, a strain with PAH inducible ovarian AHH activity. SD rats are quite resistant to PAH oocyte toxicity, suggesting that the hypothesized

relationship between the BP 3-hydroxylase and toxicity was incorrect. Using high pressure liquid chromatography techniques many BP metabolites from ovarian metabolism were isolated and identified. These experiments demonstrated that the mouse ovary can metabolize BP in the 7,8,9,10 region much more rapidly (about 5 fold) than the SD rat. Although these steps of metabolic activation (involving oxygenation, hydration and oxygenation) are only the beginning steps in the ultimate development of toxicity, they suggest that the increased toxicity observed in the mouse is the result of more rapid generation of the 7,8dihydrodiol-9,10-oxide of BP, the compound thought to be the proximate mutagen.

The study of the biochemistry of mammalian development and the regulation of differentiated functions in placenta is made possible by the availability of human first-trimester and term placental cell lines transformed by SV40 tsA mutants. The synthesis of certain proteins characteristic of differentiated human trophoblastic cells (placental alkaline phosphatase, hCG, and hCGα) can be selectively induced by changing the growth temperature of these transformed cells. The regulation of the synthesis of hCG differs in the transformed placental cells and choriocarcinoma cells. 8BrcAMP greatly induced the synthesis of hCGα in choriocarcinoma cells, the ratio of hCGα to hCG decreased (relative to the ratio in control noninduced cultures) in these cells. 8BrcAMP, therefore, induced hCG synthesis more efficiently than hCGα synthesis in choriocarcinoma cells. Alkaline phosphatase activity in choriocarcinoma cells is induced by sodium butyrate. Regulation of the induction by sodium butyrate might occur at the transcriptional level because alkaline phosphatase activity was higher (up to four-fold) in cells pretreated with sodium butyrate and cycloheximide than in cells pretreated with cycloheximide alone. Because the fetal liver plays a crucial role in embryogenesis, we have developed stable cloned lines that express the functions of differentiated liver cells under precise and controllable conditions. Normal liver cells were transformed by mutants of SV40 (tsA mutants) that are temperature sensitive in the gene required for maintenance of transformation. At the permissive temperature (33°), cells can be cultured indefinitely and readily cloned. The levels of α-fetoprotein (AFP), albumin, and transferrin are relatively low. At the nonpermissive temperature (40°), the non-transformed phenotype is regained, and the levels of these proteins are stimulated 5 to 10 fold. Most importantly, these SV40 tsA-transformed liver cells, like primary cultures of hepatocytes, respond to glucagon. In the presence of glucagon, the cAMP level is markedly elevated in the transformed cells. Thus, it appears that the glucagon receptors characteristic of hepatocytes are retained in the SV40 tsA-transformed fetal liver cells. The properties of these transformed cells indicate that they approximate normal liver cells more closely than do clones of liver cells that have been used previously. Therefore, these stable cells lines should prove advantageous in studying gene regulation and cellular differentiation in liver.

Using lipochromosomes (phospholipid entrapped metaphase chromosomes), we have transferred the human GHPRT, G6PD and PGK genes into a GHPRT deficient mouse cell line (A9). This novel technique increased the frequency of gene transfer to 1X10-5. We have now enzymatically analysed 14 of the transferent clones from two independent transfer experiments. Four clones out of 14 were analysed to see if a detectable human chromatin could be identified in their

karyotype to account for the transferred genes. The data suggest that (i) substantial human chromosomal fragments can be transferred into recipient cells using lipochromosomes; (ii) clones from human HGPRT positive A9 transferent subpopulations may or may not possess other human X-linkeg marker; (iii) the stability of lipochromosomally transferred genes is variable during continuous maintenance in the minimal essential or HAT medium; (iv) when multiple X-linked human genes are transferred a cytologically detectable human chromatin was identified in the transferents free or attached to a host chromosome; and (v) integration of transferred human chromosomal material into mouse chromosomes may occur at preferential site(s) in the recipient genome. Using the lipochromosomes (phospholipid entrapped metaphase chromosomes). We transferred the human hypoxanthine guanine phosphoribozyl transferase (HGPRT; E.C.2.4.2.8) deficient mouse cells (A9) with a frequency of approximately 1 x 10^{-5}. Two other genes located on the long arm of the human X-chromosome were also expressed in two independently derived populations of transferents (A9/GT3 or A9/GT4). We have now completed a study on the chromosomal and enzymatic composition of human HGPRT-positive clones for each subpopulation analysed in detail with alkaline Giemsa-11 staining. All of the clones expressed human phosphoglycerate kinase (PGK; EC. 2.7.2.3) and HGPRT, but one (C6) lacked human glucose-6-phosphate dephydrogenase (G6PD, EC.1.1.1.49.). In each of the clones examined, a small piece of presumptive human chromatin was visible in most cells. The chromatin fragment was free or attached in a pattern characteristic of each clone. When attached, the human chromosomal fragment appeared associated with the centrosome of the same telocentric A9 chromosome (no. 6 by X-banding) in all clones. Our findings show that substantial chromosomal fragments can be transferred into recipient cells using the lipochromosome technique. Also, integration of transferred human chromosomal material into the mouse chromosomes may occur at preferential site(s) in the recipient genome.

Availability of <u>in vitro</u> cell selective system will greatly facilitate the understanding of many genetic disorders. Unfortunately, such selective systems are very limited for mammalian cells. We have now developed a selection system for cystinotic cells. These cells due to an unknown genetic and biochemical disorder accumulate cystine in their lysosomes causing the disease, cystinosis. We found that extracellular cysteine concentrations between 0.5 and 2.5mM resulted in death of normal but not cystinotic fibroblasts grown in Eagle's Minimal Essential Medium (MEM) containing supplemental fetal calf serum and antibiotic. In co-cultivation experiments of H3-thymidine labelled cystinotic and nonradioactive normal cells, autoradiography confirmed the selective survival of cystinotic cells in medium containing 1mM cysteine. At this concentration of 1mM cysteine, intracellular cysteine content increased slightly in surviving normal but not cystinotic cells.

We reported previously that transplantation of approximately 5% of hepatic tissue from normal liver to a UDP-Glycuronyl transferase (UDPGT) deficient Gunn rat liver transfers this enzyme activity into the recipient liver. We predicted that one of the mechanisms of this induced enzyme activity may be due to the survival of a small number of normal hepatocytes in the recipient liver which produced enzyme at a 100% efficienty. To prove or disprove this hypothesis, we have done transplantation experiments with radioactively labelled donor liver. Autoradiographic study of serial hepatic sections at

different time intervals after transplantation suggest that approximately 0.9% of the transplanted cells survive in the host liver at least up to 12 weeks following grafting. The results suggest that the observed UDPGT activity in the Gunn rat after transplantation of normal liver may partially be due to these cells. Gunn rats, in the homozygons recessive state lack the enzyme Uridine disphosphoglycur only transferase (UDPGT; E.C.24.1.17). This represents an animal model for the human Crigler-Najjar syndrome. Because of the lack of this enzyme these animals, similar to their human counterpart, develop unconjugated hyperbilirubinemia. A few years ago, we were successful in measuring this enzyme activity in the enzyme deficient animals after grafting approximately 5% of normal liver tissue into the liver of the homozygons Gunn rats. We proposed in this report that the observed UDPGT activity in the Gunn rat liver following transplantation might be due in part to a small number of hepatic cells surviving in the recipient liver. To delineate (i) what proportion, if any, of the transplanted cells survived in the recipient liver and (ii) how critical was the time required for transplantation procedure in relation to the survival of these cells, we undertook an investigation, the summary of which follows: The survival of H^3-thymidine labelled normal rat hepatic cells after transplantation into the Gunn rat liver has been studied. It was found that 0.9% of the cells survived at least 12 weeks after transplantation. This suggests that the observed UDPGT activity in the genetically deficient Gunn rats, after transplantation of normal liver, may be partially due to these cells. Time required for that transplantation procedure has a profound effect on the survival of such cells in the recipient liver. The possibility of gene transfer in vivo to correct this enzyme deficiency is now being explored in our laboratory.

The process of activation of the cortical reaction is being examined in mouse, hamster and rabbit eggs in an attempt to understand the nature of activation triggered by the fertilizing sperm. Several membrane active agents, mostly ionophors, are being screened for their ability to trigger cortical reaction and, a rapid assay for the cortical reaction is being developed. Preliminary results indicate the presence of an ovoperoxidase on the surface of activated eggs. The peroxidase is believed to originate in the cortical granules and is apparently involved in the zona hardening process. Preliminary observations indicate that a peroxidase (ovoperoxidase) is released from the egg at the time of cortical reaction. The presence of this enzyme can be demonstrated using several substrates that give a color reaction in the presence of H_2O_2. The preliminary results are encouraging not only because a rapid assay will be available for release of cortical granule content, but also because demonstration of ovoperxidase will be the first direct demonstration of any enzymatic activity in the cortical granules of any species of mammalian egg. The newly developed assay for cortical granule release will be utilized in this project. Finally, we intend to pursue the isolation and characterization of the zona or sperm components involved in sperm-egg binding. Using a sperm binding assay, we will identify zona or sperm fractions which compete sperm binding to eggs.

Post-surgical adhesions are a frequent cause of failure of reconstructive surgery on fallopian tubes whose patency has been impaired. Adhesions are also a leading cause of bowel obstruction after many types of pelvic surgery. Tubal abrasions were surgically induced in 25 rhesus monkeys. Tubal patency

had been demonstrated in each animal prior to surgery. Ten of the monkeys received no post-surgical treatment. The others received various treatments designed to suppress adhesion formation. Only those monkeys treated with 32 percent dextran 70 retained tubal patency and showed no development of adhesions involving fimbria, omentum, ovary, uterus, and bladder. The 32 percent dextran 70 was introduced into the peritoneal cavity prior to closing the abdomen following the abrasion surgery. Clinical trials are now being conducted to test the apparent value of 32 percent dextran 70 in the prevention of post-surgical tubal adhesions in women.

During the development of specific and sensitive methods for the measurement of hCG, extensive studies were performed on the binding properties of an antiserum raised in the rabbit against a chemically synthesized N-acetyl triacontrapeptide corresponding to residues 116-114 of the hCG-beta subunit. Like the previously characterized antiserum to a naturally derived C-terminal fragment of the subunit, the new antiserum showed antibody recognition sites at the C-terminal pentadecapeptide region, absence of binding of ^{125}I-labeled hLH and oLH, and lack of neutralizing activity against hCG in vivo. However, the new antiserum showed higher affinity for the synthetic pentadeca- and longer peptides by comparison with the earlier antiserum, indicating that elongation of the terminal aminoacid sequence may increase antigenicity and improve the sensitivity of the specific radioimmunoassay. The previous observation that antibodies to the C-terminal peptide of hCG beta failed to inhibit the biologic activity of hCG in the rat uterine weight assay was further explored in vitro by radioreceptor assay using testis homogenates, and in vivo by studies with hCG:antibody complexes. Whereas the antibody was able to inhibit the binding of hCG to testis homogenates in vitro, administration of the preformed ^{125}I-hCG:antibody complex to male rats showed significant uptake of tracer in the testis, suggesting that hCG dissociates from the complex during circulation due to the relatively low binding constant of the interaction between hCG and the C-terminal-directed antibody. This observation provides an explanation for the apparent discrepancy between the actions of the antibody upon biological activities of hCG measured in vivo and in vitro.

Whether intra-uterine contraceptive devices (IUDs) prevent the normal progression of preimplantation events associated with blastocyst development and maintenance or cause pregnancy to fail after initiation of the implantation process is an issue of biological and social importance, in so far as the mechanism of IUD action in the human is not well understood. We reported the measurement of a urinary hCG-like gonadotropin in the late luteal phases of some IUD-bearing women by employing a highly specific radioimmunoassay system based on an antiserum (H-93) to the unique carboxyl terminal peptide (residues 123-145) of hCGβ. Further, the physical, chemical and biological properties of this hCG-like substance were examined by gel chromatography and a variety of immunological and biological tests to establish that it is an hCG-like gonadotropin distinct from typical hLH. The transitory (1-2 days) presence of a gonadotropin having immunological, biological, and physical similarity to hCG, but not typical hLH, in the late luteal phase (days 24-29) urine of some (5 of 26) IUD users was found. We did not detect hCG in sera from any of the women. Although the source of the hCG-like gonadotropin is unknown, the data suggest that late luteal phase implantation may have occurred transiently.

Reproduction research in the primate depends upon an early and reliable method for diagnosis of pregnancy. For this purpose, a hemaglutination inhibition test suitable for pregnancy diagnosis in macaques was previously developed, and distributed to primate centers throughout the world. To the present time, more than 1100 of these Subhuman Primate Pregnancy Test kits have been developed, sufficient for 55,000 pregnancy tests. The potential value of this kit was extended by a series of studies designed to determine the interval for most reliable detection of urinary chorionic gonadotropin in macaques (rhesus and cynomologus), baboons, marmosets, chimpanzees, orangutans, squirrel monkeys, tamarins, and owl monkeys. The pregnancy test yields definite results upon small volumes of urine within 2 hours, and is much more accurate and sensitive than traditional methods for pregnancy diagnosis such as palpation and bioassay.

The SPPT is useful for diagnosis of pregnancy in squirrel monkeys between 40 and 60 days in pregnancy. However, single determinations have an inherent 10% risk of false negative responses caused by low chorionic gonadotropin levels; thus, initial negative test responses should be followed within one week by an independent confirmatory test. Preliminary results with this hemagglutination inhibition test compare favorably with bioassay and may be useful, in conjunction with conventional uterine palpation, for diagnosis of pregnancy in squirrel monkeys.

We have established the interval over which urinary chorionic gonadotropin can be detected by a radioimmnoassay system during pregnancy in the golden lion tamarin. Urinary tCG was detectable in measureable quantities about four weeks after the estimated date of fertilization. Hormone levels rose to a maximum between seven and eight weeks after estimated fertilization and continued in the measurable range up to eight or nine weeks before birth. Urinary tCG was undetectable uniformly during the final two months of pregnancy. These preliminary findings on the golden lion tamarin indicate the potential value of this radioidmmunoassay system for (1) diagnosis of pregnancy by about four weeks after fertilization; (2) estimation of the excpected time of delivery; and (3) identification of individual monkeys having an apparent high risk of spontaneous abortion. The pattern of tCG excretions found here is similar to that reported earlier for other tamarin and marmoset species and squirrel monkeys, which are also New World primates.

The Subhuman Primate Pregnancy Test was evaluated as a means of detecting urinary chorionic gonadotropin to aid in pregnancy diagnosis in owl monkeys. Using radioimmunoassay, the excretion pattern of CG from pregnant owl monkeys was delineated, the hormone being detected from 16 weeks prepartum until birth. By comparison, the Subhuman Primate Pregnancy Test detected CG between week 14 prepartum and the last week of gestation with 94% accuracy. In a two year study using a simplified urine collection technique, the SPPT was shown to be a valuable procedure for diagnosing pregnancy and detecting spontaneous abortions in owl monkeys.

To study the fetal hypothalamo-hypophysial-gonadal system, the animal model chosen should appropriately allow the discirmination between fetal, placental and maternal endocrine secretions that might influence fetal gonadal development and function. The rhesus monkey is such an animal model. The

efficacy of using this primate lies in the fact that macaque chorionic gonadotrophin is not detectable in serum, urine or placental extracts beyond the sixth week in pregnancy. This leaves the fetal and the maternal pituitaries as the only apparent potential sources of gonadotrophins. Accordingly, ablation of either the fetal or maternal hypophysis thereby permits localization of the dependence of fetal gonads on secretions of the foetal or maternal pituitary. Ablation of the fetal pituitary led to a marked hypoplasia of the fetal gonads at birth. In the ovaries, the orderly progression of oogenesis had been disrupted. The percentages of normal oogonia, primordial follicles, and primary follicles were significantly reduced, and the percentages of atretic oogonia and oocytes were significantly elevated as compared to intact controls. A general hypoplasia was also observed in fetal testicular tissue after ablation of the fetal hypophysis. Testis weights were reduced three-fold, seminiferous cords were considerably less convoluted, and less extensively developed, although cord diameter remained unaltered. The intercord space was several-fold greater than for intact controls. Fetal hypophysectomy resulted in a reduction in the percentages of spermatogonia and Leydig cells at term. These observations strongly support the inference that the fetal gonads are dependent on the secretions of the fetal hypophysis during their development, especially in mid and late gestation. Depriving the fetus of its own hypophysial secretions leads to striking retardation of normal growth and differentiation of the fetal gonads with reduced survival of germ cells at birth. This retardation of normal growth is manifested, as well, in the endocrine activities of the fetal gonads.

A primate model for successful induction of intrauterine growth retardation (IUGR) as a result of depriving the fetus of approximately one-half of its viable placental mass in early and mid gestation has been described. These surrogates have significant potential for identifying the physiological and biochemical factors most critical to continued fetal growth, just when the demands of the fetus begin to surpass the functional reserve of the placenta, with attendant risks to fetal well-being. It is important that this monkey model preserves normal interactions between the maternal, placental and fetal compartments, except for the induced deficiency in functional placental tissue. Also, the surviving IUGR infant monkey has obvious apparent value in neonatal pediatric research.

The physiological control of ovarian function and endocrine regulation during the reproductive cycle in pregnancy was extensively analyzed in the monkey, as a model for understanding of ovarian function in the primate. The regulation of ovarian folliculogenesis, and selection of the dominant ovarian follicle, was examined during the primate ovarian cycle. Circulating patterns of gonadotropin and ovarian steroids were measured by radioimmunoassay before and after manipulations that included follicle cautery, luteectomy, and hemiovariectomy during the monkey menstrual cycle. In addition to these studies on follicle growth during the non-fertile menstrual cyucle, the resumption of follicle growth was investigated in these animals during the puerperium. The relationship between ovarian function and the conceptus, as well as the cessation of follicle growth during pregnancy, were also studied during the fertile menstrual cycle.

The ovarian follicle destined to ovulate was selected by the mid-follicular phase, at the expense of other follicles in the original cohort. The time-course of new follicle growth after removal of the dominant follicle was unaffected by the concurrent removal of one ovary. The next follicle destined to ovulate was not selected until after the removal fo the reigning dominant follicle. In the presence of the dominant follicle, the contralateral ovary contributes little if anything to the regulation of follicle gowth, ovulation, corpus luteum function, or gonadotropin secretion.

During the puerperium monkeys, luteectomy at term did not advance the first ovulation postpartum, and did not affect circulating gonadotropin levels. Therefore, other factors apart from the puerperal corpus luteum or the tonic level of gonadotropins must contribute to the regulation of follicogenesis in the postpartum period. Further studies in rhesus monkeys revealed that luteectomy on day 16 of pregnancy did not uniformly produce abortion, even though progesterone levels were transiently reduced to very low values. Also, bilateral ovariectomy at the same time in pregnancy was similarly followed by maintenance of the conceptus in half of the monkeys. Thus, follicle growth during the fertile cycle is inhibited by the conceptus and by some factor other than progesterone. Also, the conceptus acquires endocrine autonomy much earlier than previously believed, as early as one week after implantation.

In chronically hemiovariectomized rhesus monkeys, luteectomy was followed by a major increase in serum FSH, but the time-course of new follicle growth was unaffected. Also, no change in FSH levels occurred following luteectomy in monkeys with two ovaries. Thus, after luteectomy the two ovaries may work in concert to regulate gonadotropin secretion, but the contralateral ovary makes the predominant contribution to this process. An ovarian factor other than estradiol of progesterone appears to make a major contribution to the regulation of FSH secretion after luteectomy. Also, folliculogenesis and the negative feedback regulation of gonadotropin secretion by the ovary appear to be parallel but dissociable activities. Removal of either ovary four days after luteectomy delayed in the next LH surge by four days relative to the effect of luteectomy alone, indicating that the dominant follicle is chosen after the onset of menses but before day 8 of the cycle. These studies have further clarified asymmetrical endocrine functions of the ovaries during the menstrual cycle, and have provided evidence that the dominant ovarian follicle is selected during the first week of the menstrual cycle.

We wished to establish if the chronic absence of an ovary influenced such menstrual cycle characteristics as the lengths of the follicular and luteal phases and the attendant pattern of circulating gonadotropins and ovarian steroids. In particular, we sought to reexamine effects of previous ovarian activity on subsequent follicle growth in monkeys in which the next ovulation was contrained to occur on the same ovary that bore the corpus luteum of the previous cycle. Circulating levels of LH, FSH, estradiol, and progesterone were measured by RIA in daily serum samples throughout the menstrual cycle in five regularly cycling, chronically hemiovariectomized, adult cynomolgus monkeys. The hormonal patterns, the lengths of the follicular and luteal phases, and the overall cycle length were nearly indistinguishable form those observed in intact cycling monkeys. The findings accord with the

notions 1) that in intact monkeys, the contralateral ovary contributes little, if at all, to the regulation of the functional or lifespan of the corpus luteum, and 2) that the corpus luteum after spontaneous luteolysis has no local residual effect inimical to new follicle growth. Generalizing from these finding, it appears that in regularly cycling hemiovariectomized monkeys, constraint of follicle growth to the ovary that bore the corpus luteum of the previous cycle has no disruptive effects on folliculogenesis. Presumably, then, the corpus luteum, after its spontaneous involution, has no local, residual effects inimical to new follicle growth.

A fluorescent hCG derivative for receptor binding studies was prepared by conjugation of highly purified hCG with fluorescein isothiocynate (FITC-hCG) in a molar ratio of 3:1. The resulting FITC-hCG conjugate retained full crossreactivity with antibodies directed against intact hCG, the hCG β subunits and the carboxyl terminal peptide sequence of hCG-β. The fluorescent hormone retained receptor binding activity (85 ± 5%) and the capacity to induce progesterone production from primate luteal cells *in vitro*. After intravenous administration to female rhesus monkeys, FITC-hCG was localized within the the cell layer of certain antral follicles including the dominant follicle, but no fluorescence was noted around preantral folicles. Areas of fluorescence were also localized to discrete groups of interstitial gland cells in the stroma. The degree of fluorescence associated with these cells was indistinguishable from that observed in the theca cells. The development of a fluorescent derivative that is biologically and immunologically indistinguishable from native hCG has permitted differential microscopic localization of LH/hCG receptors in the preovulatory ovary.

Gonadotropin receptors have been localized in ovarian compartments primarily by incorporation of radiolabeled hCG. Use of fluorescein conjugates, as an alternative to radiolabeled hormones, is advantageous when *in vivo* treatment of irreplaceable animals is necessary, since large amounts of radiation contaminants can be avoided. That FITC-hCG was incorporated into ovarian tissues like native hCG was demonstrated by: 1) retention of *in vitro* biological activity of the FITC-hCG through enhancement of progesterone synthesis from collagenasedispersed luteal cells obtained from mid-luteal phase monkeys, 2) maintenance of immunological potency and parallelism between hCG and FITC-hCG in four radioimmunoassay systems, and 3) sustained FITC-hCG receptor specificity in a competive binding assay. Incubation of a constant amount of FITC-hCG with increasing concentrations of hCG decreased bound fluorescence, indicating that free unlabelled hCG competively inhibited the binding of FITC-hCG. FITC-hCG (5mcg) was given (iv) to monkeys during the follicular phase of the menstrual cycle to determine *in vivo* localization of LH/hCG receptors by fluorescent microscopy during selection of the dominant follicle. FITC-hCG was taken up by the theca cells of certain antral follicles, but especially the dominant follicle. No fluorescence was seen around preantral follicles. Additional areas of fluorescence were associated with interstitial glands. In order to increase the number of follicles selected for pre-ovulatory status, mild hyperstimulation was achieved by giving monkeys hMG for 3 day intervals (cycle days 1-3 or 4-6) prior to injection of FITC-hCG. By day 7 of the cycle, it was apparent that the uptake of FITC-hCG was preferentially associated with "selected" follicles both by intrafollicular fluorescence, and by that of the surrounding theca and interstitial glands.

In conclusion, the FITC-hCG conjugate retained the biological and immunological characteristics of native hCG. In addition, use of fluorescent labeled hCG permitted identification of the presemptive dominant follicle, before it was recognizable by light microscopy.

To investigate the paradoxical actions of hCG on ovarian function, 1000 IU hCG was administered to monkeys during 16 menstrual cycles in the late follicular phase, either on day 9 or 11 after the onset of menses. Retrospectively, hormonal profiles indicated that 6 of 16 hCG treatments were given coincident with incipient gonadotropin surges. In the remaining 10 cycles, hCG was given prior to the initiation of spontaneous gonadotropin surges. No untoward effects of hCG were observed in monkeys treated coincident with/or after the onset of spontaneous FSH/LH surges. That is, cyclic ovarian function continue unabated, with the interval between spontaneous gonadotropin surges being 28 $\pm$ 2.0 days (mean $\pm$ SE). In the 10 cycles having hCG injected prior to initiation of endogenous gonadotropin surges, induced atresia of the expected dominant follicle was presumed from the absence of the anticipated midcycle elevations of serum estradiol-17B, LH and FSH, and lack of a luteal phase rise in progesterone. Resumption of cyclic ovarian function was delayed significantly. Surprisingly, in 5 of these 10 monkeys, FSH concentrations increased about 3-fold within one week and persisted for the duration of the study (> 3 weeks). Among these 5 monkeys, the concentrations of LH, estradiol and progesterone were indistinguishable from the remaining 5, in which serum FSH concentrations did not change appreciably. The data indicate that, after the initiation of the preovulatory gonadotropin surges, the ovaries are not susceptible to these pharmacologic impairments induced by hCG. In contrast, when administered prior to initiation of the spontaneous FSH and LH surges, hCG induced 1) apparent atresia of the dominant follicle, 2) arrest of cyclic ovarian function lasting more than 3 weeks, and 3) in 5 of 10 monkeys, an altered modulation of tonic FSH secretion, independent of LH release, where serum FSH persisted at levels about 3-fold above normal.

To differentiate hypothalamic-pituitary from ovarian factors responsible for post-gestational supression of folliculogenesis, 28 rhesus monkeys were studied following either surgically-induced abortion 21 days after fertilization, or spontaneous delivery at term. Ovarian refractoriness was evaluated by administration of low doses of hMG to stimulate follicular maturation. Alternatively, estradiol benzoate was injected to test for induction of gonadotropin surges. Resumption of spontaneous ovulations was delayed until 38.4 $\pm$ 5.7 (mean $\pm$ S.E.) days after induced abortion, whereas sham abortion did not deter cyclic ovarian function in nonpregnant monkeys. That folliculogenesis had been stimulated by hMG, both post-abortion and post partum, was confirmed by the presence of multiple preovulatory follicles on both ovaries (N=3-7/ovary), as assesed by lapatotomy, and by concurrent elevations of serum estradiol-17β. hCG therapy caused final maturation and rupture of all or most of these preovulatory follicles within 24h as indicated by the development of multiple stigma, and subsequent increases in serum progesterone levels. The interval from abortion to induced ovulation was 12.5 $\pm$ 1.3 days; and from delivery until induced ovulation was 12.2 $\pm$ 1.8 days. Resumption of estradiol benzoate stimulated gonadotropin surges was not apparent until 21 days post-abortion or until 35 days after full-term delivery. These findings demonstrate that pregnancy does not impart any

apparent refractoriness to gonadotropin stimulation upon the ovaries directly, but rather, establishes a gestational (duration-dependent) suppression of hypothalamic-pituitary function. Although tonic gonadotropin secretion may also be inadequate to support folliculogenesis post partum, responsivity to the positive feedback actions of estrogen on FSH and LH surges was impaired transiently for about 5 weeks.

To evaluate the functional integrity of the hypothalmic-pituitary system in post-gestational monkeys, peripheral serum concentrations of LH and FSH were determined in post partum (nursing and non-nursing) and post-abortion (mid-pregnancy) females challenged with estradiol benzoate at a dose known to be effective in eliciting gonadotropin surges in cycling monkeys. In a longitudinal study of nursing the non-nursing post partum mothers and post-abortion females, estradiol benzoate was administered serially during weeks 1, 3, 5, 7, 9, and 11 post-delivery. Some nursing monkeys were challenged with estradiol benzoate up to 6 months post partum. In a cross-sectional study of nursing mothers, this response to estradiol benzoate was evaluated during weeks 1, 3, 5, 7, and 9 post partum. Hypothalamic-pituitary responsivity to estradiol benzoate was impaired in nursing monkeys through 180 days post partum, in that FSH or LH surges were not induced by estrogen therapy. In contrast, non-nursing post partum mothers and post-abortion females regained their sensitivity to this positive feedback action of estrogen within 35 to 49 days. Our findings indicate that during gestation a transient "lesion" develops in the hypothalamic-pituitary system and persists into the puerperium. It blocks the positive feedback response of surge gonadotropin secretion induced by estradiol. However, this hypothalamic-pituitary failure is not sufficient by itself to explain post partum infertility in these primates, since in non-nursing monkeys spontaneous resumption of ovulatory menstrual cycles seldom occurs until 2 to 4 months after delivery at term.

When circulating gonadotropin levels are elevated to achieve ovulation induction by enhancing endogenous secretions (clomiphene citrate) or following hMG injections in either women or monkeys, multiple/bilateral ovulations are common. Thus, the elevated levels of gonadotropins reaching the ovaries can override the natural processes of follicular recruitment and selection which normally generate a single ovulation each menstrual cycle. Accordingly, supra-physiologic levels of gonadotropins can increase the number of follicles to be ovulated either by increasing the number recruited into maturational growth or be preventing fated atresia or both. hMG was administered to intact, cycling adult cynomolgus monkeys in the early (day 1-3), mid (days 4-6), or late (days 8-10) follicular phase to determine the effects of elevated serum gonadotropins, as a function of time in cycle, on the number of follicles stimulated to grow to the secretion of estradiol. In the presence of the dominant follicle, the responsiveness of other follicles within both ovaries to the stimulatory effects of hMG was markedly suppressed. Even following ablation of the dominant follicle, hMG therapy was associated with diminished ovarian responsiveness to gonadotropins, with a significant delay in resumption of new follicle growth.

Initiation of normal, ovulatory menstrual cycles in post-menarchial monkeys requries a functionally mature hypothalamic pituitary ovarian axis. To examine the maturation of this axis, the regulation of gonadotropin secretion

by hypothalamic and ovarian factors was assessed in infant and juvenile rhesus and cynomolgus monkeys. Serial serum samples were collected from intact monkeys between birth and 10 mon and between 28 and 48 months during the same intervals the monkeys were evaluated for responsiveness to GnRH. To study feedback regulation by the ovary, monkeys were either castrated at birth or at 2 1/2 yr to evaluate negative feedback, or treated with estradiol benzoate as infants or juveniles to assess the actions of estrogen on the surge and tonic modes of gonadotropin secretion. FSH and LH concentrations in peripheral plasma were determined by RIA. In intact infants, tonic levels of FSH and LH fluctuated markedly. Tonic levels of FSH and LH in juvenile monkeys were less variable. Peak concentrations of FSH and LH following GnRH treatment were greater in infants than in juveniles: 2-4 fold vs 1-2 fold increases above tonic levels. In infants castrated at birth, serum FSH rose above intact levels, reaching adult castrate levels by 1 1/2 mon before declining to intermediate concentrations at 6 mon. In contrast, LH levels increased slightly only during the first month. After castration at 2 1/2 year, FSH and LH rose within the first month to adult castrate levels. In neither intact infants nor juveniles did estradiol benzoate elicit gonadotropin surges, but in infants estradiol benzoate did transiently suppress serum FSH from 10.8 $\pm$ 2.0ug/ml to undetectable concentrations. These findings indicate that the hypothalamic-pituitary-ovarian axis is functional in both infant and juvenile monkeys, but it does not respond in an adult fashion to castration or estrogen feedback. Though the tonic secretion of gonadotropins in infants and juveniles was quantitatively adult-like, FSH and LH secretion of neonates was particularly responsive to GnRH. Thus, maturation of the hypothalamic-pituitary-ovarian axis in these primates involves developmental changes in the regulation of both the tonic and surge modes of gonadotropin secretion.

An examination of the serum concentrations of luteinizing hormone, estradiol-17β, and progesterone throughout the menstrual cycle of the stumptailed monkey (Macaca arctoides) indicated that the secretory pattern of these hormones was similar to that of the rhesus monkey. Serum concentrations of FSH throughout the menstrual cycle in the stumptailed macaque show a pattern similar to that reported for other species of monkeys and the woman, except for notable FSH elevations 2-3 days after the preovulatory gonodotropin surges in 6 of 9 cases.

Recently, it was found that charcoal-treated bovine and procine follicular fluid (PFF1) reduced peripheral plasma FSH levels, with little alteration in LH levels, in gonadectomized rats and in cycling female rats and monkeys. The data suggested the presence of a nonsteroidal substance (inhibin) in follicular fluid of thought to be responsible for regulation of FSH secretion. Administration of porcine follicular fluid to suppress circulating FSH offers a convenient, novel system for evaluating the actions of FSH during the ovarian cycle. In current studies we are testing the effects of PFF1 administration during the first half of the menstrual cycle on follicle development in the rhesus monkey. The goal of this research is to test the hypothesis that the formation and steroidogenic activity of luteal tissue may be impaired, when the corpus luteum originates from a developing follicle exposed to less-than-normal FSH.

That ovarian follicular fluid contains non-steroidal substances (inhibin) which selectively suppress FSH secretion both *in vivo* and *in vitro* is well established. However, evidence that inhibin is a normal secretory product of ovarian origin which modulates FSH secretion by the pituitary during the normal menstrual cycle has not been presented. Accordingly, in monkeys concommitant collections of ovarian venous blood, in the presence of the dominant follicle (largest visible follicle on cycle days 9-11), analogous peripheral blood from a femoral vessel, and antral fluid from the dominant follicle, were made for comparisons of inhibin-like activity *in vitro*. Although addition of either ovarian venous serum or follicular fluid, collected in direct association with the dominant follicle, did suppress FSH secretion selectively in the pituitary culture system, whereas peripheral serum did not, this affect could be due to steroidal as well as non-steroidal components. For example, both the fluid within the dominant follicle and the adjacent venous blood are estrogen rich. However, that the secretion of LH was unaltered by the addition of the same specimens which markedly suppressed FSH release suggests selective action of inhibin constituents of ovarian vein blood and follicular fluid of monkeys during the late follicular phase. Continuing studies are aimed at distinguishing between steroidal and non-steroidal effects of these fluids as a function of time in the menstrual cycle.

The regulation of corpus luteum function in the primate was further studied by utilizing an *in vitro* system of isolated luteal cells from the rhesus monkey. The ability of such cells from cycling or pregnant animals to produce progesterone *in vitro* was a function of the stage of the menstrual cycle and gestation. Cells from the mid-luteal phase of the menstrual cycle produced the greatest amounts of progesterone, and were the most responsive to gonadotropic stimulation with hCG. Luteal cells from early pregnancy secreted little progesterone, and were unresponsive to hCG. However, with advancing gestation, luteal cells again acquired the capacity for steroidogenesis, and at term were similar to mid-luteal phase cells in their basal and hCG-stimulated levels of progesterone production. It appears likely that prolonged exposure of the coupus luteum to high levels of gonadotropin during early pregnancy is responsible for the refractoriness to further gonadotropic stimulation *in vitro*. Addition of estradiol was found to antagonize the steroidogenic effect of hCG in isolated luteal cells, consistent with the ability of estradiol to cause functional regression of the primate corpus luteum *in vivo*.

For *in vitro* studies on the cellular mechanisms that regulate luteal function and luteolysis, a relatively prolonged culture period is required. To this end, a method for the dispersion and isolation of monkey granulosa lutein cells was developed, by collagenase dispersion and culture in Ham's F10 medium. Progesterone production declined markedly between day 2 and day 6, but remained detectable until day 10 and was responsive to hCG throughout the ten days of culture. Two distinct cell populations were identified in the cultured luteal cells, with respect to size and fine morphological structure. These will be separated from each other and contaminating cells prior to future studies on cultured luteal cells, designed to find the growth characteristics and steroidogenic properties of homogeneous cell populations derived from the corpus luteum.

To assess the refractory state of the rhesus monkey corpus luteum following 2-3 weeks exposure to endogenous macaque chorionic gonadotropin (mCG) in early pregnancy, we investigated in vitro the effect of hCG, dibutyryl cyclic AMP (dbcAMP) and pregnenolone on progesterone production by luteal cells isolated from monkeys during early pregnancy (Days 22-29 of gestation). For comparison, luteal cells obtained during the menstrual cycle (Days 16-19) and at term pregnancy (Days 160-165 of gestation) were also studied. Under control conditions, luteal cells obtained on Days 22-24 of pregnancy produced significantly less progesterone than cells from midluteal phase of the menstrual cycle, despite little difference in the weight of corpora lutea excised. The data indicated that the gonadotropin refractory state of the rhesus monkey corpus luteum following prolonged exposure to mCG in early pregnancy may include an impairment of the gonadotropin receptoradenylyl cyclase system in luteal cells and a reduction in enzyme activity necessary for progesterone synthesis. The refractory condition of the corpus luteum appears to be reversible since gonadotropin-sensitive progesterone production is characteristic of rhesus monkey luteal cells at term pregnancy.

To assess the hypothesis that luteolysis in primates may occur as a consequence of estrogen secretion by the corpus luteum, estradiol, estrone, and progesterone production was examined in suspensions of luteal cells isolated form corpora lutea of rhesus monkeys at various stages of the menstrual cycle. Under basal conditions, day 16-19 cells produced significantly greater amounts of estradiol than estrone whereas day 21-22 cells synthesized similar quantities of these estrogens due to a reduction in estradiol production. Basal progesterone estrone production by day 16-19 and day 21-22 cells was similar. In the presence of hCG estradiol and progesterone production by day 21-22 cells was significantly less than that by day 16-19 cells. However, hCG-stimulated estrone production remained unchanged. Day 23-25 cells produced significantly less progesterone, estradiol and estrone than day 16-19 cells can failed to respond to hCG. Incubation with testosterone or androstenedione markedly increased estrogen sytnehsis by day 16-19 cells without altering progesterone secretion. Dihydrotestosterone did not affect luteal cell estrogen production. Day 16-19 cells and day 21-22 cells produced primarily estradiol during incubation with testosterone. Androstenedione enhanced both estradiol and estrone production by day 16-19 cells. However, by day 21-22 of the cycle, luteal cells secreted primarily estrone during incubation with androsteneidione. Exposure to pregnenolone (1000 ng/ml), but not progesterone, enchanced luteal cell estradiol and estrone production in a manner similar to that by androsteroidione. The addition of gonadotropin failed to enchance estrogen production in the presence of testosterone or androsteneidione. The data provide <u>in vitro</u> evidence of the capacity of luteal cells from the rhesus monkey to synthesize and secrete estrogen as well as progesterone. The estrogen synthetic capacity of luteal cells did not increase around the time of luteolysis and decreased coincident with declining progesterone secretion. However, luteal cell conversion of precursors to estradiol and esterone indicates that changes in the level or form of estrogen in the corpus luteum <u>in vivo</u> could occur just prior to luteolysis due to midulation of the aromatizing enzyme system or the availability of androgen in luteal cells.

In vitro assessment of rhesus monkey (Macaca mulatta) luteal cell function was further extended utilizing dissociated cells from the corpus luteum. The main topics of interest in this project have included 1) morphological and functional correlations of luteal cells maintained in culture for ten days, 2) Effects of non-gonadotropin hormones and cholesterol on the maintenance of functional luteal cells, 3) evaluation of progesterone production by dissociated luteal cells after brief exposure to trypsin, and 4) localization of LH/hCG receptors of dissociated luteal cells.

Simulated menstrual cycles were produced in 20 castrate monkeys by sequential treatment with estradiol and progesterone in silastic capsules. Radioimmunoassay of estratiol and progesterone, and gonadotropins in peripheral serum provided assuredness of the hormonal status of each monkey under treatment. Cytosol and nuclear receptors for these steroids were measured in the endometrium after different intervals of the treatment. Estradiol receptor levels were not changed during the estrogen sequence, but were lowered by progesterone therapy in both cytosol and nuclear components. Progesterone receptor synthesis in cytosol was induced by exogenous estrogen. The total concentration of progesterone receptor decreased with the uptake of progesterone by the cell; meanwhile, the ratio of cytosol to nuclear progesterone receptors declined. These data suggested that this sequential estrogen-progesterone regimen induces the changes in steroid receptor patterns in endometrium of ovariectomized monkeys which occurs due to ovarian cyclicity in the normal menstrual cycle.

As illustrated by the studies summarized in this report, the investigators within the Pregnancy Research Branch have developed a broad range of studies on normal processes and disorders which affect fertility and gestation. A variety of animal models and clinical interests are being pursued with the expectation that useful information will be derived about the basic causes and optimal treatment of disorders adversely affecting the reproductive process, especially pregnancy.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00026-04 PR

PERIOD COVERED March 1, 1979 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Fertilization and Activation of Development in Mammals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Bela J. Gulyas	Senior Investigator	PRB, NICHD
Other:	Eli D. Schmell	Staff Fellow	PRB, NICHD
	Lydia C. Yuan	Chemist- Technician	PRB, NICHD

COOPERATING UNITS (if any)

NONE

LAB/BRANCH Pregnancy Research Branch

SECTION

INSTITUTE AND LOCATION NICHD, NIH Bethesda, MD 20205

TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.4	OTHER: 0.1

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The process of activation of cortical reaction is being examined in mouse, hamster and rabbit eggs in an attempt to understand the nature of activation triggered by the fertilizing sperm. Several membrane active agents, mostly inophors, are being screened for their ability to trigger cortical reaction and, a rapid assay for the cortical reaction is being developed. Preliminary results indicate the presence of an ovoperoxidase on the surface of activated eggs. The peroxidase is believed to originate in the cortical granules and is apparently involved in the zona hardening process.

Objective

This research project has been resumed in March 1979. The objectives of the proposed project are threefold: (1) determine how the sperm triggers cortical reaction and activation of development at the time of fertilization of mammalian eggs; (2) define the molecules involved in the species - specific sperm-egg recongnition; (3) elucidate the mechanisms involved in the cortical reaction and the resultant alterations in the egg cell surface, such as zona hardening, zona reaction and block to polyspermy.

Methods to be employed

There is an urgent need to develop a rapid, reliable assay for determining cortical reaction in mammalian eggs. Preliminary observations indicate that a peroxidase (ovoperoxidase) is released from the egg at the time of cortical reaction. The presence of this enzyme can be demonstrated using several substrates that give a color reaction in the presence of H_2O_2. The preliminary results are encouraging not only because a rapid assay will be available for release of cortical granule content, but also because demonstration of ovoperoxidase will be the first direct demonstration of any enzymatic activity in the cortical granules of any species of mammalian egg. Activities of other enzymes, such as proteases, esterases, phosphatases and glycosidases will also be tested.

Upon determination of the presence of any of the above enzymes we shall pursue determining the role of the cortical granule enzymes in the alteration of the zona pellucida and the egg plasma membrane surface, during fertilization.

Cortical granules apparantly carry a net negative charge which keeps them out of contact with the negatively charged inner surface of the plasma membrane. It is now recognized that Ca^{2+} is generally required for the release of secretory products of diverse cells. Several membrane active agents, primarily inophores, will be screened for their ability to trigger cortical reaction. The newly developed assay for cortical granule release will be utilized in this project.

Finally, we intend to pursue the isolation and characterization of the zona/or sperm components involved in sperm-egg binding. Using a sperm binding assay, we will identify zona or sperm fractions which compete sperm binding to eggs.

Significance of Proposed Work

The long-range goal of the research proposal outlined above, is to understand the biochemical mechanism(s) of sperm-egg interactions and the mode of activation of development at fetilization.

The importance of cortical reaction is better appreciated when one considers the abnormal conditions that often occur in eggs. When cortical reaction is not elicited by the fertilizing sperm (first sperm to enter), then accessory sperm can penetrate the egg, as it was demonstrated by the PI, in the rabbit. Polyspermy leads to abnormal chromosomal numbers which is usually lethal to the

embryo. The failure of cortical reaction could be due to either the spermatozoa that lack the capacity to trigger cortical reaction (thus it might be considered as a form of male infertility), or alternatively, to the egg, which, for some reason, fails to respond to the activation of the sperm. It is now known that the cortical granules of eggs aged in vivo or in vitro undergo abnormal changes and fail to demonstrate normal cortical reaction. It would be of importance to know if cortical granules of eggs from animals (or man) near termination of their fertile life span, demonstrate similar abnormal changes.

Another form of abnormal cortical reaction can occur in an event that the cortical granules are released prior to penetration of the sperm. In this case fertilization cannot occur, because the zona pellucida, or the plasma membrane of the egg, blocks entry of the sperm. Indeed, there has been one report stating that in the eggs of some women the cortical granules are released premaurely, potentially rendering these eggs unfertilizable. Precocious release of cortical granules is yet to be proven as a form of human infertility. However, should it be the case, first we must understand the nature of activation of development by the sperm before we can learn how to handle the abnormalities.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00029-05 PR

PERIOD COVERED October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

In Vitro Studies on Primate Luteal Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B.J. Culyas	Senior Investigator	PRB	NICHD
Other:	G.D. Hodgen	Chief, (Acting)	PRB	NICHD
	L.C. Yuan	Chemist - Technician	PRB	NICHD

COOPERATING UNITS (if any)

LAB/BRANCH Pregnancy Research Branch

SECTION Section on Endocrinology

INSTITUTE AND LOCATION NICHD, NIH Bethesda, MD 20205

TOTAL MANYEARS: 2.5	PROFESSIONAL: 0.5	OTHER: 2

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In vitro assessment of rhesus monkey (Macaca mulatta) luteal cell function was further extended utilizing dissociated cells from the corpus luteum. The main topics of interest in this project have included 1) Morphological and functional correlations of luteal cells maintained in culture for ten days, 2) Effects of non-gonadotropin hormones and cholesterol on the maintenance of functional luteal cells, 3) Evaluation of progesterone production by dissociated luteal cells after brief exposure to trypsin, and 4) Localization of LH/hCG receptors of dissociated luteal cells.

PHS-6040
(Rev. 10-76)

Objectives

Studies on the regulation of corpus luteum CL function in subhuman primates were further extended this year. In the monkey (Macaca mulatta) the CL of nonfertile menstrual cycle is a transient organ as indicated by the decline in the levels of circulating progesterone (P) and regression of luteal cells toward the end of the menstrual cycles. The process of functional regression of the primate corpus luteum in the menstrual cycle is not well understood, yet it remains an important determinant in fertility management. The objectives of this study were to: 1) develop a primary culture system for dissociated luteal cells whereby their steroidogenic activity can be maintained; 2) improve the dissociation procedure enabling separation of different size luteal cells; and 3) localize LH/hCG receptors on the surface of dissociated luteal cells.

Methods Employed

To facilitate studies on the cellular mechanisms that regulate both luteal function and luteolysis, we developed a long-term tissue culture system for dissociated monkey luteal cells. Our initial approach has been to ask whether cytodifferentiation, as assessed by accumulation of P and the presence of fine structural properties characteristic of steroidogenic luteal cells, can be maintained in the absence of the intact animals' milieu. Originally, luteal cells were dispersed from corpus luteum of cycling monkeys (Macaca mulatta) by collagenase digestion (0.2% collagenase in F10-HEPES medium) at 37°C in an atmosphere of 95% O_2:5% CO_2 with constant shaking. The enzymatic digestion was followed by gentle mechanical disruption. This procedure yielded single cells and cell clusters. The earlier dissociation procedure was improved by employing Ca^{+2}-free medium, EDTA, DNase and trypsin (TR) in one or more steps of the dissociation procedure. A short exposure (10 min) of luteal cells to trypsin enhanced the yield of single cells. However, since information was lacking on the ability of TR-treated dispersed primate luteal cells to respond to gonadotropins, we examined this question. As a last step of the cell dissociation procedure, the mixtures of single cells and cell clusters were incubated in the presence of TR for 10 min. At the end of this pre-incubation period cells were washed free of the pre-incubation medium, then incubated for 3h in the presence or absence of hCG (CR 119). Alternatively, dissociated luteal cells were incubated for 3h in the continuous presence of TR as well as hCG. Lastly, we wanted to determine whether TR during the continuous treatment of the previous experiment effects cell receptors or destroys the hCG. In group A, dissociated luteal cells were pre-incubated in the presence of TR for 1h, then washed and incubated with hCG for 3h. In group B, cells were not pretreated. Instead, the hCG was incubated in the presence of TR for 1h. Then, luteal cells were incubated in the presence of the TR-treated hCG for 3h. Proper controls accompanied the experiments.

Studies were also initiated to localize LH/hCG receptors on the surface of dissociated luteal cells. A conjugate of hCG (CR119) to horseradish peroxidase (HRP) was used as a probe in these experiments. The HRP-hCG conjugate, prepared by Drs. H. Chen and S. Matsuura, had 1% and 0.024% biological activity as determined by the radioreceptor assay and the testosterone assay, respectively. The HRP-hCG had 0.25 peroxidative activity of the original HRP. A con-

centration of 10µg/ml of HRP-hCG was used in the experiments in order to equate the gonadotropin activity with 100ng of hCG (a concentration previously found to have maximal effect on progesterone secretion).

Major Findings

In our initial study we found that monkey luteal cells can be maintained in vitro and they produce detectable amounts of P for up to 10 days, although P production declined markedly after four days in culture. Although these cells remained sensitive to hCG ($P<0.01$), the general pattern of diminished P production could not be reversed. Cells cultured in F10 for 6 days before exposure to hCG increased P production ($P<0.01$).

In order to make this system adaptable to test any luteolytic agent, the levels of P production by cells in culture must be sustained at a higher level for a longer period of time. In an effort to achieve this goal we further modified our culture conditions. Preliminary observations indicated that addition of insulin (I), cortisol (C) and thyroxin (T) to the culture medium, along with hCG, resulted not only in higher initial levels of P production, but also in higher P levels throughout the 10-day culture period. Nevertheless a general diminishing pattern of P productions occurred just as it occurred without the addition of I, T, C. The addition of cholesterol, a metabolic intermediate of steroidogenesis, enhanced P production to a lesser extent than I, T, C in the presence of hCG, and again, a diminishing P production was observed.

Collagenase dispersion of monkey CL yields single cells and cell clusters At least two populations of luteal cells were identified with respect to size (15-25 µm and 30-45 µm) and fine structural characteristics. Several attempts were made to separate the different size luteal cells, but these results were hampered by the cell clusters. We have improved the dissociation procedure using Ca^{+2}-free medium, EDTA, DNase and typsin, in one or more steps of the dissociation procedure. As the last step in the dissociation procedure, the mixture of single cells and cell clumps were incubated in the presence of TR for 10 minutes. The brief exposure of luteal cells to TR had no effect on the basal levels of P production during a 3-h incubation period. Furthermore, luteal cells that were pre-incubated with TR responsed to hCG stimulation with increased P production, and the response to hCG was independent of TR concentration. However, continuous exposure (3h) of cells to TR significantly ($P<0.01$) depressed basal P secretion and inhibited the response to hCG. TR has no effect on the biopotency of hCG. We conclude that short term (10 min) exposure of monkey luteal cells to TR had no adverse effect on their ability to secrete P in vitro. Since the functional integrity of LH/hCG receptors of these cells were maintained, TR can be used in the dissociation procedure.

The (HRP-hCG) conjugate stimulated P secretion in a linear fashion during 3 hours of incubation. The low peroxidative activity of the conjugate, however, hampered systematic, repeatable localization of HRP-hCG on the cell surface. Internalization of the conjugate occurred only when the cells were exposed to HRP-hCG at 37°C. Under these conditions internalization of the bound conjugate occurred within a few minutes. Before this system can be a

useful test in probing gonadotropin receptors, the peroxidative activity of the conjugated HRP must be improved.

Significance

We have shown that primate luteal cells can be maintained in tissue culture system and can be utilized to study luteolysis. The improved dissociation procedure does not alter the cells ability to produce steroids and respond to exogenous gonadotropins. Since functional regression of the primate corpus luteum remains an important determinant in fertility management and contraceptive action, the in vitro system can be utilized to answer some of the important questions concerning the physiological life-span of the corpus luteum.

Proposed Course

If monkey CL remains available for in vitro studies we shall use the recently improved dissociation procedure to obtain single cell population of luteal cell. Once accomplished, we will renew our efforts to isolate the different size luteal cells and define their growth characteristics and steroidogenic potential in our in vitro system.

Publications:

1. Gulyas, B.J., R.L. Stouffer and G.D. Hodgen: Progesterone Synthesis and Fine Structure of Dissociated Monkey (Macaca mulatta) Luteal Cells Maintained in Culture. Biol. Reprod., 20:779-792.

2. Gulyas, B.J., L.C. Yuan and G.D. Hodgen: Progesterone Production by Dispersed Monkey (Macaca mulatta) Luteal Cells After Exposure to Trypsin. Steroids: (Submitted).

3. Gulyas, B.J., L.C. Yuan and D.G. Hodgen: The Effects of non-gonadotropic Hormones and Cholesterol in the In Vitro Maintenance of Monkey (Macaca mulatta) Luteal Cells (Submitted).

4. Stouffer, R.L., Nixon, W.E., and Hodgen, G.D.: Disparate Affects of Prostaglandins on Basal and Gondodotropin - stimulated Progesterone Production by Luteal Cells Isolated from Rhesus Monkeys During the Menstrual Cycle and Pregnancy. Biol. Reprod. 20: 897-906, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00030-06 PR

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Characterization of Patterns of Chorionic Gonadotropin Production in Primates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	G.D. Hodgen	Chief (Acting)	PRB	NICHD
Other:	C.K. Turner	Biologist Technician	PRB	NICHD
	D.L. Barber	Biologist Technician	PRB	NICHD

COOPERATING UNITS (if any)

LAB/BRANCH: Pregnancy Research Branch

SECTION: Section on Endocrinology

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

As described in previous reports, a hemagglutination inhibition test suitable for the diagnosis of pregnancy in macaques has been developed in this laboratory. The Subhuman Primate Pregnancy Test Kit, distributed under the aegis of the Contraceptive Development Branch, Center for Population Research, NICHD, has been sent to primate facilities all over the world. To date, more than 1100 kits have been delivered, sufficient for more than 55,000 pregnancy tests. In order to realize the full potential of this it, we have undertaken a series of studies to determine the interval for most relilable detection of urinary chorionic gonadotropin from a variety of primtes including: macaques (Rhesus and Cynomolgus), baboons, marmosets, chimpanzees, orangutans, squirrel monkeys, tamarins, and owl monkeys.

PHS-6040
(Rev. 10-76)

Project Description: Z01 MD 00030-06 PR

The need for early, reliable diagnosis of pregnancy in primates derives from their importance as surrogates for man in biomedical research where the earliest possible diagnosis of pregnancy is required, such as investigations of teratogenic against contraceptive evaluation and improving the efficiency in breeding management situations.

Urine specimens were collected form tamarins and owl monkeys at various intervals throughout pregnancy. Using the Subhuman Primate Pregnancy Test Kit, these specimens were tested in this hemagglutination inhibition test to determine the interval during which investigators could excpect a reliable diagnosis of pregnancy.

Pregnant tamarins excrete chorionic gonadotropin at detectable levels from day 35 until near 100 days in gestation: owl monkeys from 14-130 days in pregnancy. As these results illustrate, the Subhuman Primate Pregnancy Test Kit is a valuable laboratory tool for use with a variety of species. However, its proper application requires careful testing of each type of primate because of widely different patterns of chorionic gonadotropin excretion. Application of these new findings will have 2 points of significant impact: 1) these monkeys are the only useful laboratory species for malaria research and their export from South American has ceased, necessitating domestic breeding; 2) detection of pregnancy by palpation causes a high rate of fetal wastage. The SPPT avoids handling the monkey to achieve pregnancy diagnosis.

By comparison with traditional procedures for diagnosis of pregnancy in primates, including palpation and bioassay, this hemagglutination inhibition test is obviously the method of choice. This test yields definitive results within 2 hours, requires only 2 drops of neat urine, and can be conducted adjacent to the animal quarters without elaborate instrumentation.

Publications:

1. Hodgen, G.D., Kleiman, D.G., and Gracey, D.L.: Levels in Pregnancy Golden Lion Tamarins Urinary Chorionic Gonadotropin J. Med. Primatology 7: 333-338, 1978.

2. Hodgen, G.D. and Hall, R.D.: Pregnancy Diagnosis in Owl Monkeys (Aotus trivivgatus): Evaluation of the Hemagglutination Inhibition Test for Urinary Chorionic Gonadotropin. Lab. Anim. Sci. 29: 345-351, 1979.

3. Hodgen, G.D., Stolzenberg, S.J., Jones, D.C.L., Barth, R.A. and Kaplan, J.N.: Studies with Timed-pregnant Squirrel Monkeys (Saimiri sciuveus). J. Med. Primatology 8: 29-38, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00031-06 PR

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Hormonal Interrelationships between Fetus, Mother and Placenta in Primates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	G.D. Hodgen	Chief (Acting)	PRB	NICHD
Other:	B.J. Gulyas	Senior Investigator	PRB	NICHD
	R.F. Williams	Staff Fellow	PRB	NICHD
	G.S. diZerega	Clinical Associate	PRB	NICHD
	M.L. Yonekura	Guest Worker	PRB	NICHD
	E.G. Lundblad	Clinical Associate	PRB	NICHD

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Pregnancy Research Branch

SECTION

Section on Endocrinology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XXX☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Over the past eight years, a continuum of studies has been directed at understanding the hormonal interrelationships between the fetal, placental and maternal compartments. Most of these studies have utilized primate models, where the similarities to equivalent events in human pregnancies are most extensive. During this past year, we concluded a series of experiments which employed fetal hypophysectomy in rhesus monkeys to establish the importance of fetal hypophysical secretion for gonadal development in utero; particular attention was given to testicular development.

PHS-6040
(Rev. 10-76)

Project Description: Z01 HD 00031-06 PR

Objectives

In new, but related investigations we have: 1) begun to characterize the evolution of fetal gonadal receptors for LH/hCG using florcein-labelled hCG; 2) analyzed fetal response to GnRH as a function of fetal age; 3) used ligation of transplacental vessels to induce intrauterine growth retardation, and 5) measured prolactin in maternal and placental blood after fetectomy to determine the primary origin of this hormone in the mid and late gestational milieu.

Major Findings

We conclude that a dynamic interaction of fetal and maternal hypothalamic-pituitary axes governs the development of the fetus and its endocrine organs. Survival of gametes within the fetal ovaries and testes of these primates is dependent primarily on secretions of the fetal pituitary. Preliminary results suggest that the monkey fetus responds to GnRH injection with increased secretion of FSH and LH only during the perinatal interval in utero.

We have evidence that the placental reserve is directly related to intrauterine growth retardation and is manifest after a progressive loss of functional placental tissue.

Proposed Course

Having learned that secretions of the fetal pituitary are essential to normal growth and development of ovaries and testes in utero in rhesus monkeys, we now set out to examine the secretory capacities of the fetal and newborn monkey hypophysis. Specifically, we will begin by characterization of FSH and LH concentrations in fetal and infant serum at various "ages." Tests will be conducted to examine both negative and positive feedback conditions which regulate gonadotropin secretion in utero.

Current studies will show the evolution of fetal gonadal receptors for LH/hCG during organogenesis. Also, injections of GnRH agonists and antagonists will be used to examine functional dynamics of the hypothalamic-pituitary-gonadal axis at various gestational ages. Finally, studies to monitor fetal stress acutely after exceeding the placental reserve will lead us toward early diagnosis of intrauterine growth retardation.

Publications:

1. Gulyas, B.J., Tullner, W.W., and Hodgen, G.D.: Fetal or Maternal Hypophysectomy in Rhesus Monkeys (Macaca mulatta): Effects on the Development of Testes and Other Endocrine Organs. Biol. Reprod. 17: 650-660, 1978.

2. Hodgen, G.D., Primate Models for Pregnancy Hormone Secretion in Women: Fetal, Maternal and Placental Factors. (Chapter 32) Animal In Models for Contraceptive and Fertility Research, ed. Alexander/Lippincott, 1979.

3. Gulyas, B.J., Research Review: The Hypothalamo-Hypophysial-Gonadal System in Fetal Rhesus Monkeys. Research in Reproduction 10: 3-4, 1978.

4. Williams, R.F., Johnson, D.K., Hodgen, G.D. Ovarian Estradiol Secretion During Early Pregnancy In Monkeys: Luteal Versus Estra-Luteal Secretion and Effect of Chorionic Gonadotropin. Steroids 32: 539-545, 1978.

5. Graham, C.E. and Hodgen, G.D. Use of Chimpanzees in Reproductive Biology. J. Med. Primatology 8: 000-000 1979.

6. Goodman, A.L. and Hodgen, G.D. Corpus Luteum-conceptus-follicle Relationships During the Fertile Cycle in Rhesus Monkeys: Pregnancy Maintenance Despite Early Luteal Removal. J. Clin. Endocrinol. Metab. , 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00045-04 PR

PERIOD COVERED October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Regulation of Folliculogenesis in the Monkey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Gary D. Hodgen	Chief (Acting)	PRB	NICHD
OTHER:	Wilbert E. Nixon	Senior Investigator	PRB	NICHD
	Arnold L. Goodman	Staff Fellow	PRB	NICHD
	Robert F. Williams	Staff Fellow	PRB	NICHD
	Gere S. diZerega	Clinical Associate	PRB	NICHD

COOPERATING UNITS (if any)

LAB/BRANCH Pregnancy Research Branch

SECTION Section on Endocrinology

INSTITUTE AND LOCATION NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 4	PROFESSIONAL: 2	OTHER: 2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Endocrine factors contributing to the regulation of folliculogenesis (selection of the dominant follicle) during the primate ovarian cycle were examined in two non-human primate models, the rhesus monkey, and the cynomolgus monkey. Circulating patterns of gonadotropins and ovarian steroids (estradiol and progesterone) were monitored by radioimmunoassay before and after follicle cautery, luteectomy and hemiovariectomy during the menstrual cycle in monkeys. In addition to examining follicle growth during the nonfertile menstrual cycle, the resumption of follicle growth was studied during the puerperium. Ovary-conceptus interrelationships, as well as the cessation of follicle growth in pregnancy were studied during the fertile menstrual cycle. Salient findings include: 1) usefulness of the cynomolgus monkey as alternative non-human primate model, 2) asymmetrical function of the two ovaries during the menstrual cycle, 3) a dual stimulatory/inhibitory role for macaque chorionic gonadotropin, 4) evidence that the dominant follicle is selected during the first week of the menstrual cycle, and 5) evidence that FSH secretion is regulated by an ovarian factor(s) other than estradiol or progesterone.

PHS-6040
(Rev. 10-76)

Objectives

To examine the regulation of follicle growth during the primate ovarian cycle, its cessation during pregnancy, and its resumption post partum, using non-human primate models. To examine functional capacities of the hypothalamic-pituitary-ovarian axis in neonates.

Methods

Circulating patterns of gonadotropins and ovarian steroids were determined by radioimmunoassay before and after ablation of the cyclic structure (dominant follicle, or corpus luteum), or one ovary (hemiovariectomy), performed at laparotomy. GnRH challenges, castrates and steroid therapy of neonates has been undertaken. Also, adult cycling monkeys have been stimulated with hMG and clomid to examine the role of gonadotropins in follicle recruitment and selection.

Major Findings

In the primate ovarian cycle the follicle destined to ovulate in a given menstrual cycle is selected already by the mid-follicular phase, and at the expense of any other follicles of the original lot. The time course of new follicle growth after removal of the dominant follicle is unaffected by the concurrent removal of one ovary. The next follicle destined to ovulate was not selected until after the removal of the reigning dominant follicle. In the presence of the dominant follicle, the contralateral ovary contributes little, if at all, to the regulation of follicle growth, ovulation, corpus luteum function, or gonadotropin (its ablation goes "unnoticed"). Unlike bilateral ovulators, the occurrence of a "compensatory" rise in gonadotropin release after unilateral ovariectomy in this primate is conditional as the ovary removed and the surety of the dominant follicle.

Follicle growth is arrested during the luteal phase, and the follicle growth culminating in ovulation is unaffected by the concurrent removal of one ovary. The next follicle destined to ovulate is not selected until after the removal (demise) of the corpus luteum. In the presence of the corpus luteum, the contralateral ovary contributes little, if at all, to the regulation of gonadotropin secretion, follicle growth, or corpus luteum function (i.e., its removal goes "unnoticed"). Unlike bilateral ovulators, the monkey uses its ovaries in sequence, rather than in concert, and a compensatory response to unilateral ovariectomy during the luteal phase is conditional on the ovary removed, and the surety of the corpus luteum.

Constraining ovulation and corpus luteum function to one ovary has no disruptive effects on folliculogenesis. Thus, the corpus luteum, after spontaneous involution, has no local, residual effects inimicable to new follicle growth.

Two potential determinants of the resumption of folliculogenesis post partum, the rejuvenated corpus luteum, and the tonic supply of gonadotropins,

were examined in nonsuckling monkeys during the puerperium. Luteectomy at term did not advance the first ovulations post partum circulating levels of gonadotropins were similar to levels observed during pregravidic ovulatory cycles. Thus, some other factor(s) apart from the puerperal corpus luteum, or the tonic supply of gonadotropins, contribute to the regulation of folliculogenesis post partum.

Luteetomy in chronically hemiovariectomized rhesus monkeys was followed by a major increase in serum FSH, but the time-course of new follicle growth was unaffected compared to monkeys bearing two ovaries; no change in FSH levels occurred in monkeys with two ovaries, even though the opposite ovary was wedge-resected at luteectomy. Thus, following luteectomy (but not before), the ovaries may work in concert to regulate gonadotropin secretion, but the contralateral ovary (even after wedge-resection) makes the preponderant contribution. An ovarian factor other than estradiol of progesterone made a major contribution to regulating the secretion of FSH after luteectomy. In addition, folliculogenesis and the negative-feedback regulation of gonadotropins by the ovary appear to the parallel, dissociable activities.

That pregnancy creates a natural hiatus in folliculogenesis is well established. Whether this involves ovarian refractoriness to gonadotropic stimulation or suppression of hypothalamic-pituitary function has been debated at length. Our findings, using hMG/hCG therapy during mid-gestation, post partum, after early abortion and during the spontaneous luteal phase all indicate that the ovaries retain responsiveness to moderate levels of FSH and LH. We conclude that the hormonal milieu of pregnancy blocks folliculogenesis at some point(s) in the hypothalamic-pituitary system.

Among neonatal female monkeys, we found that GnRH caused an abrupt rise in FSH secretion, but a lesser increase in LH release. By 6 months of age, exogenous GnRH was barely able to effect any increase in FSH or LH. Preliminary results with the RICT in vitro bioassay indicate that the bio vs. immuno potencies of serum LH are discrepant, emphasizing the danger of relying exclusively, on RIA hormonal values.

When either ovary was removed 4 days after luteectomy the next LH surge was delayed by 4 days relative to luteectomy alone. Thus the dominant follicle is chosen after the onset of menses, but before Day 8 of the cycle.

Significance to Biomedical Research

The determination of the time of the selection of the dominant follicle (to within a week), and the elucidation of the asymmetrical activities of the ovaries during the menstrual cycle will be of vital concern to clinicans working to enhance (or suppress) fertility in women.

Proposed Course

When induction of ovulation is important clinically, two major problems are now accessable: 1) how to avoid multiple ovulations during clomid or

hMG therapy and the inherent risk in such pregnancies and 2) how to avoid premature follicular or instititial luteinization from excessive LH treatment in the follicular phase. The result is high serum progesterone and refractoriness to gonadotropins. These problems will receive intense interest in the coming year.

Publications

1. Goodman, A.L., and Hodgen, G.D., Menstrual Cycle Characteristics in Chronically Hemiovariectomized Cynomolgus Monkeys (macca fascicularis) J. Clin. Endocrinol. Metab. 48: 345, 1979

2. Goodman, A.L., and Hodgen, G.D. Between-ovary interaction in the regulation of follicle growth, corpus luteum function, and gonadotropin secretion in the primate ovarian cycle. I. Effects of follicle cautery and hemiovariectomy during the follicular phase in cynomolgus monkeys. Endocrinology 104: 1304, 1979.

3. Goodman, A.L., and Hodgen, G.D. Between-ovary interaction in the regulation of follicle growth, corpus luteum function, and gonadotropin secretion in the primate ovarian cycle. II. Effects of lutuctomy and hemiovariectomy during the luteal phase in cynomolgus monkeys. Endocrinology 104: 1310, 1979.

4. Goodman, Arnold L., Nixon, Wilbert E., and Hodgen, Gary D. Between-ovary interaction in the regulation of follicle growth, corpus luteum function, and gonadotropin secretion in the primate ovarian cycle. III. Temporal and spatial dissociation of folliculogenesis and negative feedback regulation of gonadotropin release after luteectomy in rhesus monkeys. Endocrinology 105: 69, 1979.

5. diZerega, Gere S., and Hodgen, Gary D. Pregnancy Associated Ovarian Refractoriness to Gonadotropin: A Myth. Am. J. Ob. Gyn. 134: 819, 1979.

6. Williams, Robert F., Johnson, David K., and Hodgen, Gary D. Resumption of estrogen-induced gonadotropin surges in post partum monkeys. J. Clin. Endocrinol. Metab. 49: , 1979.

7. diZerega, Gere S., Williams, Robert F., Morin, Martin L., and Hodgen, Gary D., Anovulation Following Pregnancy Termination: Ovarian vs Hypothalamic Pituitary Factors. J. Clin. Endocrinol. Metab. , 1979.

8. Goodman, Arnold, L. and Hodgen, Gary D. Corpus Luteum-conceptus-follicle Relationships During the Fertile Cycle in Rhesus Monkeys: Pregnancy Maintenance Despite Early Luteal Removal. J. Clin. Endocrinol. Metab. , 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00177-02 PR

PERIOD COVERED: October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Cynomolgus Monkey (Macaca fascicularis) as an Appropriate Surrogate

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	G.D. Hodgen	Chief (Acting)	PRB	NICHD
Other:	R.F. Williams	Staff Fellow	"	"
	D.L. Barber	Biological Laboratory Technician	"	"
	C.K. Turner	Biological Laboratory Technician	"	"
	D.K. Johnson	Chief, Veterinary Medicine	VRB	DRS

COOPERATING UNITS (if any)

Veterinary Resources Branch, DRS

LAB/BRANCH: Pregnancy Research Branch

SECTION: Section on Endocrinology

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2	PROFESSIONAL: 1	OTHER: 1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Effective April 1, 1978, India banned the export of rhesus monkeys. In October and December of 1978 we were able to get about 60 rhesus monkeys from Bangladesh. Now this source too is gone. This primate occupies a totally unique place in biomedical sciences research, in that perhaps 90% of the information known about reproduction in non-human primates has been learned from this species. Since domestic production of rhesus monkeys will not meet demands in the laboratory, a suitable substitute primate, with broad utility must be found. Also, a huge amount of baseline study must be conducted to establish the appropriateness of this new species as a surrogate for the rhesus monkey. As reported a year ago, the cynomolgus monkey appears to be the best substitute for the rhesus. Continuting efforts confirm this perspective.

PHS-6040
(Rev. 10-76)

Project Description: Z01 HD 00177-02 PR

Our observations strongly suggest that the cynomolgus monkey (Macaca fascicularis) is now the primate of choice. We have characterized pituitary gonadotropin and ovarian steroid secretion in the menstrual cycle of cynomolgus monkeys, determined chorionic gonadotropin levels and steroid output of placental origin throughout gestation. In addition, we have described the patterns of LH, FSH, estradiol and progesterone in the post partum interval through reinitiation of cyclic ovarian function. During the past year, sufficient baseline data have accumulated to warrant heavy investment in these primates by application of cortical investigative protocols. This has provided very significant relief from the shortage of rhesus monkeys. Many laboratories around the U.S.A. and abroad have followed this lead.

Publications:

1. Williams, R.F., Johnson, D.K., and Hodgen, G.D.: Ovarian Estradiol Secretion During Early Pregnancy in Monkeys: Luteal Versus Extra-Luteal Secretion and Effect of Chorionic Gonadotropin. Steroids 32: 539-546, 1978.

2. Hodgen, G.D. Primate Models for Pregnancy Hormone Secretion in Women: Fetal, Maternal and Placental Factors. (Chapter 32) In: Animal Models for Contraceptive and Fertility Research, Alexander ed., Holden Day, Inc.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00900-01 PR

PERIOD COVERED: October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Factors Regulating Estrogen and Progesterone Receptors in Monkey Endometrium in the Fertile Menstrual Cycle

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	G. D. Hodgen	Chief (Acting)	PRB	NICHD
Other:	B. Kreitmann	Guest Worker	PRB	NICHD
	O. Kreitmann	Guest Worker	PRB	NICHD

COOPERATING UNITS (if any)

LAB/BRANCH: Pregnancy Research Branch

SECTION: Section on Endocrinology

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 3	PROFESSIONAL: 2	OTHER: 1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Appropriate methods for repeated surgical collection of endometrial tissue from monkeys and characterization of cytosol and nuclear estrogen and progesterone receptors have been developed. Equilibrium dissociation constants of estradiol and progesterone were 2.1 x 10^{-10}M and 3.6 x 10^{-9}M, respectively. When estrogen or estrogen + progesterone replacement therapy was given to castrate monkeys, we found that progesterone receptor synthesis was induced by estrogens.

PHS-6040
(Rev. 10-76)

Objectives

To develop surgical and other methodologic procedures for studying factors influencing endometrial receptors for estrogens and progesterone in monkeys. To investigate the action of these receptors in facilitating implantation. To exploit these findings for the purposes of fertility regulation, either to resolve infertility or provide contraception.

Methods

At abdominal transfundal hysterotomy, we were able to collect endometrial tissue repeatedly. Standardized techniques used characterization of steroid receptors in other species were adapted to optimal circumstances. By giving estrogen and progesterone replacement therapy to castrate monkeys in a pattern which mimicks the normal ovulatory menstrual cycle, follicular phase and luteal phase factors influencing receptor formation were evaluated.

Major Findings

Simulated menstrual cycles were produced in 20 castrate monkeys by sequential treatment with estradiol and progesterone in silastic capsules. RIA of E_2 and P, and gonadotropins in peripheral serum provided assuredness of the hormonal status of each monkey under treatment. Cytosol and nuclear receptors for E_2 and P were measured in the endometrium after different intervals of the treatment. E_2 receptor (E_2R) levels were not changed during the estrogen sequence, but were lowered by progesterone therapy in both cytosol and nuclear components. Progesterone receptor (PR) synthesis in cytosol was induced by exogenous estrogen. The total concentration of PR decreased with the uptake of P by the cell; meanwhile, the ratio of cytosol to nuclear P receptors declined. These data suggest that this sequential estrogen-progesterone regimen induces the changes in E_2R and PR patterns in endometrium of ovariectomized monkeys which occurs due to ovarian cyclicity in the normal menstrual cycle.

Proposed Course

Current studies center on the changes in endometrial steroid receptors that acompany implantation in the fertile menstrual cycle and the abnormal characteristics of endometrial receptors for these steroids during short luteal phase infertility.

References

Kreitmann-Gimbal, B., Kreitmann, O.L., Bayard, F. and Hodgen, G.D., Characterization of Estrogen and Progesterone Receptors in Monkey Endometrium: I. Surgical and Biochemical Methodology. Steroids 00: 000-000, 1979.

Kreitmann-Gimbal, B., Kreitmann, O.L., Bayard, F. and Hodgen, G.D., Characterization of Estrogen and Progesterone Receptors in Monkey Endometrium: II. Effects of Estradiol and/or Progesterone on Endometrium of Castrate Monkeys. Steroids 00: 000-000, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00901-01 PR

PERIOD COVERED: October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Core Endocrine Laboratory Unit

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Wilbert E. Nixon,	Senior Investigator	PRB, NICHD
Other:	Rudolph Reid		PRB, NICHD
	Jerrie Shewbridge		PRB, NICHD

COOPERATING UNITS (if any)

LAB/BRANCH: Pregnancy Research Branch

SECTION: Section on Endocrinology

INSTITUTE AND LOCATION: NICHD, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	3.0	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Core Endocrine Laboratory Unit moved into renovated quarters during June, 1979. A number of activities are in progress or remain available in support of several NICHD research and clinical programs. Research interest within the unit are determined by NICHD basic and clinical research protocols, where existing resources either among current laboratory arrangements or contracting services, are not adequate to support or expand essential activities. The core lab also trains clinical associates and various visiting scientists in methods of hormone assays.

PHS-6040
(Rev. 10-76)

1. Sheep anti-rabbit gamma globulin serum (2nd antibody) was produced at the NIH farm, titered for potency, stored and distributed to NICHD investigators. Sheep plasmapheresis enhanced yield per bleed, reduced stress to animals and will lead to a reduction in number of sheep required for 2nd antibody production. Approximately 25 liters of this antiserum with a commercial value in excess of $200,000 was distributed to NICHD investigators. Antiserum potency was determined using as assay containing 10-fold more normal rabbit serum than that utilized by many laboratories, therefore, potency undoubtedly exceeded that of commercially available reagents.

2. Urinary free cortisol determinations were provided in support of NICHD clinical investigations. Approximately 600 determinations. Involving both individual samplings and series of samplings for patient care and investigations were processed. Extensive investigations of conditions for LH-20 chromatography led to a reliable radioimmunoassay which compared favorably with results obtained by a commercial firm when samples were run in parallel.

3. Quantal primate chrionic gonadotropin determinations of pregnancy during the breeding for monkeys were provided for PRB programs and collaborative investigations on reservation and for collaborative investigations at off-reservation sites. The ovine luteinizing hormone beta subunit antiserum and labeled human chorionic gonadotropin system was one previously developed in PRB. The assay has an apparent response to chorionic gonadotropin only and, therefore highly predictive of pregnancy in primates.

4. Specific human chorionic gonadotropin radioimmunoassays (hCGB RIA) were performed in support of research and clinical investigations associated with NICHD programs. The time course of administered hCG in serum of both primate and rodent were among results generated with this procedure. Patient care requirements are quite modest at present with regard to this RIA, however, a continuing capability is maintained.

5. Rat luteinizing hormone (rLH) and Follicle-Stimulating Hormone (rFSH) radioimmunoassays were performed on a continuing basis in support of NICHD programs as well as research with-in this unit. Radioimmunoassays (RIA) for rLH were carried out weekly or more often depending on requirements. Results of investigations involving stimulation of pituitary cell cultures to produce LH or the determination of time course of LH production/secretion during perfusion experiments utilizing various stimuli are among the data generated. Reagents used were those supplied by the National Pituitary Agency and supported by the NIAMDD.

The addition of Jerrie Shewbridge to the laboratory made this procedure possible with respect to the level of activity.

6. A rapid estradiol radioimmunoassay (E2 R RIA) was developed to permit same day estrogen determination. This procedure has been employed extensively in research protocols involving the timing of ovulation and induced ovulation and induced ovulation in primates. An apparently high degree of correlation exists between follicular maturation as observed at laparoscopy and serum estradiol as determined by this assay procedure. Daily determinations on

numerous specimens and utilizing appropriate controls has demonstrated a high degree of reliability for the procedure. Non-human primate research and clinical programs share support from the rapid E-2 radioimmunoassay.

7. Cyclic AMP and Cyclic GMP radioimmunoassays have been established in the laboratory utilizing commercial kits (New England Nuclear, Amersham). The level of activity for this procedure is dependent on the needs of the NPM Branch, NICHD.

Projected course:

Unit capability to aid on-going NICHD programs is being monitored and support for clinical and collaborative investigations is being provided. Projects involving nonhuman primates and some involving clinical applications are being pursued. The rapid estrogen assay is essential in the timing of hormonal or other intervention in these investigations. This acute procedure will be made available as requirements dictate and as personnel availability allow.

Quantal assays for primate chorionic gonadotropin to monitor pregnancy during the breeding season are to be provided as required.

The immature rat, hypophysectomized and intact, will be utilized in in vestigations of the possible influence of micronutrients (vitamins), protein and calorie availability on ovarian steroidogenesis. In addition, the influence of steroid metabolites on the aromatase complex as reflected in estrogen production will be studied. Administered gonadotropins will allow evaluations in the basal and stimulated states. In vitro organ culture production of steroids will be determined in tissue from control and treated animals.

Additional laboratory activities will involved utilizing specific assay procedures found in this report in support of NICHD programs and development of others in collaboration with NICHD investigators with specific requirements.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00902-01 PR

PERIOD COVERED: October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Developmental Enzymology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	J. Chou	Senior Investigator	PRB, NICHD
	T. Mano	Visiting Fellow	PRB, NICHD
	S. Schlegel	Visiting Fellow	PRB, NICHD

COOPERATING UNITS (if any)

LAB/BRANCH: Pregnancy Research Branch

SECTION

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 3.0	PROFESSIONAL: 3.0	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Topics of current interest are: (1) the regulation of the synthesis of placental proteins, alkaline phosphatase and human chorionic gonadotropin in normal placenta, choriocarcinoma and SV40-transformed placental cells. (2) Regulation of differentiated functions (synthesis of α-fetoprotein, albumin and transferrin) in liver. (3) Hormonal regulation of glycogenolysis and glyconeogenesis in liver.

PHS-6040
(Rev. 10-76)

Project Description: Z01 HD 00902-01 PR

Objectives: The main objectives of this project are, (1) to study cellular differentiation and gene regulation in placenta and liver, (2) to isolate and characterize selected enzymes and other proteins from normal placenta choriocarcinoma cells, and (3) to characterize the hormonal regulation of glycogenolysis and glyconeogenesis in liver.

Methods Employed: The principal methods employed are: (1) protein isolation techniques, including salt- and solvent-fractionation, gel-filtration, ion-exchange chromatography, and affinity chromatography; (2) enzyme assays, which are usually based on spectrophotometric or radiometric methods, (3) radioimmunoassays or hormones, liver proteins, and cAMP; and (4) transformation of placental cells and liver cells with SV40.

Major Findings: (1) The study of the biochemistry of mammalian development and the regulation of differentiated functions in placenta are made possible by the availability of the SV40 tsA mutants-transformed human first-trimester and term placental cell lives. The synthesis of certain proteins characteristic of differentiated human trophoblastic cells, placental alkaline phosphatase, hCG, and hCGα was studied in the transformed placental cells and in choriocarcinoma cells. 8BrcAMP greatly induced the synthesis of hCGα in the transformed first-trimester and term placental cells with little or no stimulation of the synthesis of hCG. The ratio of hCGα to to hCG in the transformed placental cells, therefore, increased in the presence of 8BrcAMP. On the other hand, 8BrcAMP induced the synthesis of hCG as well as hCGα in choriocarcinoma cells. In the presence of 8BrcAMP, the ratio of hCGα to hCG decreased (relative to the ratio in control noninduced cultures) in choriocarcinoma cells. 8BrcAMP, therefore, induced hCG synthesis more effeciently than hCGα synthesis in choriocarcinoma cells. Our data indicate that the regulation of the production of hCG differs in the two types of placental cells. (2) Alkaline phosphatase activity in choricocarcinoma cells is induced by sodium butyrate. Regulation of the induction by sodium butyrate might occur at the transcriptional level because alkaline phosphatase activity was higher (up to four-fold) in cells pretreated with sodium butyrate and cycloheximide than in cells pretreated with cycloheximide alone. (3) Because the important role of fetal liver in embryogenesis, we have now obtained stable cloned lines that express the functions of differentiated liver cells under precise and controllable conditions. Normal liver cells were transformed by mutants of SV40 (tsA mutants) that are temperature sensitive in the gene required for maintenance of transformation. At the permissive temperature (33°), cells can be cultured indefinitely and readily cloned: the levels of α-fetoprotein (AFP), albumin, and transferrin are relatively low. At the nonpermissive temperature (40°), the transformed phenotype is lost, and the levels of these proteins are stimulated 5 to 10 fold.

Most importantly, these SV40 (tsA) transformed liver cells, like primary cultures of hepatocytes, respond to glucagon. In the presence of glucagon, the cAMP level is markedly elevated in the transformed cells. Thus, it appears that the glucagon receptors characteristic of hepatocytes are retained in the SV40 (tsA) transformed fetal liver cells.

The properties of these transformed cells indicated that they approximate normal liver cells more closely than do other clones of liver cells that have

been used previously. Therefore, these stable cell lines should prove advantageous in studying gene regulation and cellular differentiation in liver.

Significance to Biomedical Research and the Program of the Institute: The study of the mechanisms of mammalian cell differentiation is of importance becuase it leads to understanding normal cellular function and may provide insights into abnormal biological states.

The investigations of hormonal regulation and gene expression in placenta and liver are essenleatial for an understanding of fetal development and embryogenesis. The study of the control of gene expression of alkaline phosphatase, hCG, and AFP is of importance because these substances are derepressed in many tumors. Ectopic production of these fetal proteins may serve as an early signal of malignant transformation. The establishment of human placental cells and rat fetal liver cells which approximate normal placental and liver cells in vivo respectively should help us to elucidate the control mechanisms for the synthesis of these proteins.

Proposed Course of Project: Continued exploration of (1) the eutopic and ectopic induction of alkaline phosphatase and hCG in these trophoblast systems and in nontrophoblastic tumor cells. (2) Regulation of thes synthesis of α-fetoprotein (AFP) albumin and transferrrin in the liver cells. (3) Characterize hormonal response in liver cells.

Publications:

1. Chou, J.Y.: Regulation of synthesis of human chorionic gonadotropin by strains of HeLa cells in culture. In Vitro 14: 775-778, 1978.

2. Chou, J.Y.: Gene regulation in placenta: A model system using human placental cells transformed by tsA mutants of simian virus 40 in Carcino-Embryonic Proteins, Chemistry, Biology, Clinical Application, Frank-Gunter Lehmann Editor, Elsevier/North-Holland Volume 2, 1979 p. 643-650.

3. Sakiyama, T., Robinson, J.C., and Chou, J.Y.: Multiple forms of alkaline phosphatase in untreated and 5-bromo-2'deoxyuridine-treated choriocarcinoma cells. Arch. Biochme. Biophys. 191, 782-791, 1979.

4. Sakiyama, T., Robinson, J.C., and Chou, J.Y.: Characterization of alkaline phosphatase from human first-trimester placentas. J. Biol. Chem. 254, 935-938, 1979.

5. Chou, J.Y.: Regulation of the induction of alkaline phosphatase in choriocarcinoma cells by sodium butyrate. In Vitro, 1979, (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD00903-01 PR

PERIOD COVERED: July 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Enzyme induction in a genetically deficient animal by normal hepatic tissue transplantation.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.: A. B. Mukherjee, Senior Investigator, PRB/NICHD

Other: B. Foley - Chemist- Technician " "

A. Agrawal - Visiting Fellow " "

COOPERATING UNITS (if any)

NONE

LAB/BRANCH: Pregnancy Research Branch

SECTION:

INSTITUTE AND LOCATION: NIH, NICHD Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We reported previously that transplantation of approximately 5% of hepatic tissue from normal liver to a UDP-Glycuronyl transferase (UDPGT) deficient Gunn rat liver transfers this enzyme activity into the recipient liver. We predicted that one of the mechanisms of this induced enzyme activity may be due to the survival of a small number of normal hepatocytes in the recipient liver which produced enzyme at a 100% efficiency. To prove or disprove this hypothesis we have done transplantation experiments with radioactively labelled donor liver. Autoradiographic study of serial hepatic sections at different time intervals after transplantation suggest that approximately 0.9% of the transplanted cells survive in the host liver at least up to 12 weeks following grafting. The results suggest that the observed UDPGT activity in the Gunn rat after transplantation of normal liver may partially be due to these cells.

PHS-6040
(Rev. 10-76)

Gunn rats, in the homozygons recessive state lack the enzyme Uridine disphosphoglucuronyl transferase (UDPGT; E.C.24.1.17). This represents an animal model for the human Crigler-Najjar syndrome. Because of the lack of this enzyme these animals, similar to their human counterpart, develop unconjengated hyperbilirubinemia. Few years ago we were successful in measuring this enzyme activity in the enzyme deficient animals after grafting approximately 5% of normal liver tissue into the liver of the homozygons Gunn rats (Mukherjee & Krasner [1973], Science, 182: 68). We proposed in this report that the observed UDPGT activity in the Gunn rat liver following transplantation might be due in part to a small number of hepatic cells surviving in the recipient liver. To delineate (i) what proportion, if any, of the transplanted cells survived in the recipient liver and (ii) how critical was the time required for transplantation procedure in relation to the survival of these cells, we undertook an investigation, the summary of which follows: The survival of H^3-thymidine labelled normal rat hepatic cells after transplantation into the Gunn rat liver has been studied. It was found that 0.9% of the cells survived at least 12 weeks after transplantation. This suggests that the observed UDPGT activity in the genetically deficient Gunn rats after transplantation of normal liver may be partially due to these cells. Time required for that transplantation procedure has a profound effect on the survival of such cells in the recipient liver.

The possibliity of gene transfer in vivo to correct this enzyme deficiency is now being explored in our laboratory.

Publications

1. Mukherjee, A.B. and Krasner, J., 1979, Survival of transplanted normal hepatic cells in bilirubin UDP-Glucuronyl transferase deficient Gunn rat liver Res. Commun. Chem. Path. Pharmacol. 24:159-168.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00904-01 PR

PERIOD COVERED
July 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Chromosome mediated gene transfer in mammalian cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I. A. B. Mukherjee, Senior Investigator, PRB/NICHD
Other: B. Foley - Chemist- Technician, " "
A. Agrawal - Visiting Fellow " "

COOPERATING UNITS (if any)

None

LAB/BRANCH
Pregnancy Research Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Using lipochromosomes (phospholipid entrapped metaphase chromosomes) we have transferred the human HGPRT, G6PD and PGK genes into a HGPRT deficient mouse cell line (A9). This novel technique increased the frequency of gene transfer to 1X10-5. We have now enzymatically analysed 14 of the transferent clones from two independent transfer experiments. Four clones out of 14 were analysed to see if a detectable human chromatin could be identified in their karyotype to account for the transferred genes. The data suggest that (i) substantial human chromosomal fragments can be transferred into recipient cells using lipochromosomes; (ii) clones from human HGPRT positive A9 transferent subpopulations may or may not possess other human X-linked markers; (iii) the stability of lipochromosomally transferred genes is variable during continuous maintenance in the minimal essential or HAT medium; (iv) when multiple X-linked human genes are transferred a cytologically detectable human chromatin was identified in the transferents free or attached to a host chromosome; and (v) integration of transferred human chromosomal material into mouse chromosomes may occur at preferential site(s) in the recipient genome.

PHS-6040
(Rev. 10-76)

Using lipochromcsomes (phospholipid entrapped metaphase chromosomes). We transferred the human hypoxanthine guanine phosphoribosyl transferase (HGPRT; E.C.2.4.2.8) deficient mouse cells (A9) with a frequency of approximately 1 x 10^{-5} (Mukherjee et al., 1978, Proc. Natl. Acad. Sci, U.S.A. 75: 1361-1365). Two other genes located on the long arm of the human X-chromosome were also expressed in two independently derived populations of transferents (A9/GT3 or A9/GT4). We have now completed a study on the chromosomal and enzymatic composition of human HGPRT-positive clones for each subpopulation analysed in detail with alkaline Giemsa-11 staining.

All of the clones expressed human phosphoglycerate kinase (PGK; EC. 2.7.2.3) and HGPRT, but one (C6) lacked human glucose-6-phosphate dephydrogenase (G6PD, EC.1.1.1.49.). In each of the clones examined, a small piece of presumptive human chromatin was visible in most cells. The chromatin fragment was free or attached in a pattern characteristic of each clone. When attached, the human chromosomal fragment appeared associated with the centrsome of the same telocentric A9 chromosome (no. 6 by Q-banding) in all clones.

These data suggest that (a) substantial chromosomal fragments can be transferred into recipient cells using the lipochromosome technique, and (b) integration of transferred human chromosomal material into the mouse chromosomes may occur at preferential site(s) in the recipient genome.

We are now attempting, in collaboration with Dr. Judith Levin, to transfer oncogenic proviral genome from one cell to another using the lipochromosome technique.

Publications

1. Mukherjee, A. B., Orloff, S., Butler, J. DeB., Triche, T., Lalley, P. and Schulman, J. D. 1978. Entrapment of metaphase chromosomes into phospholipid vesicles (lipochromosomes): Carrier potential in gene transfer. Proc. Natl. Acad. Sci. U.S. 75:1361-1365.

2. Hoffman, W. K., Lalley, P., Butler, J. DeB., Orloff, S., Schulman, J. D. and A. B. Mukherjee, 1979, Lipochromosome mediated gene transfer: Identification and probable specificity of localization of human chromosomal material in the transferents. Proc. Natl. Acad. Sci. U.S.A. (submitted for publication).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HI 00905-01PR

PERIOD COVERED July 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Development of in vitro selective system for cystinotic cells.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.: A. B. Mukherjee, Senior Investigator, PRB/NICHD
Other: B. Foley - Chemist- Technician " "
A. Agrawal - Visiting Fellow " "

COOPERATING UNITS (if any)

NONE

LAB/BRANCH Pregnancy Research Branch

SECTION

INSTITUTE AND LOCATION NIH, NICHD Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER XXX

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Availability of in vitro cell selective system will greatly facilitate the understanding of many genetic disorders. Unfortunately, such selective systems are very limited for mammalian cells. We have now developed a selection system for cystinotic cells. These cells due to an unknown genetic and biochemical disorder accumulate cystine in their lysosomes causing the disease, cystinosis. We found that extracellular cysteine concentrations between 0.5 and 2.5mM resulted in death of normal but not cystinotic fibroblasts grown in Eagle's Minimal Essential Medium (MEM) containing supplemental fetal calf serum and antibiotic. In co-cultivation experiments of H3-thymidine labelled cystinotic and nonradioactive normal cells, autoradiography confirmed the selective survival of cystinotic cells in medium containing 1mM cysteine. At this concentration of 1mM cysteine, intracellular cysteine content increased slightly in surviving normal but not cystinotic cells. This resistance of cystinotic fibroblasts to elevated extracellular cysteine forms the basis for an in vitro selective system for these mutant cells.

Nephropathiscystinosis is an autosomal recessive disease characterized by lysosomal accumulation of L-cystine in most body tissues. The nature of the primary biochemical defect has yet to be elucidated. While working with cultured fibroblasts derived from cystinotic patients, we discovered that cysteine at certain extracellular concentrations is toxic to normal but not cystinotic fibroblasts, thus providing a selective system for cystinotic cells. Extracellular cysteine concentrations between 0.5 and 2.5 mM resulted in death of normal but not cystinotic cells grown in Eagle's Minimal Essential Medium (MEM) containing supplemental fetal calf serum and antibiotic. In co-cultivation experiments of ^{3}H-thymidine labelled cystinotic with nonradioactive normal fibroblasts, autoradiography confirmed the selective survival of cystinotic cells in medium containing 1 mM cysteine. At this concentration of 1mM cysteine, intracellular cysteine content increased slightly in surviving normal but not in cystinotic cells. This resistance of cystinotic fibroblasts to elevated extracellular cysteine concentrations forms the basis for an *in vitro* selective system for these mutant human cells. Further exploration of this resistance phenomenon may well expand the understanding of the molecular defect in cystinotic cells. These studies are now being conducted in collaboration with the laboratory of Dr. Joseph Schulman.

Publication

1. Orloff, S., Mukherjee, A.B., Butler, J. DeB., Foley, B., and J.D. Schulman, 1979, Cystinotic and normal fibroblasts: Differential suscaptibility to cysteine toxicity *in vitro*. Proc. Natl. Acad. Sci. U.S.A. (submitted for publication).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00168-03 PR

PERIOD COVERED: October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Ovarian Xenobiotic Metabolism and Oocyte Toxicity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Donald R. Mattison	Senior Investigator	PRB	NICHD
Other:	Maria Nightengale	Chemist- Technician	PRB	NICHD
	Kenji Shiromize	Visiting Fellow	PRB	NICHD
	Bela J. Gulyas	Senior Investigator	PRB	NICHD

COOPERATING UNITS (if any)

R. H. Menard, Senior Investigator, LMC, NCI
D.W. Nebert, Chief, DPb, NICHD
S.S. Thorgeirsson, Senior Investigator, LCHP, NCI

LAB/BRANCH: Pregnancy Research Branch

SECTION:

INSTITUTE AND LOCATION: NICHD, NIH Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The rodent ovary contains the microsomal monooxygenases capable of metabolizing polycyclic aromatic hydrocarbons to reactive intermediates. The polycyclic aromatic hydrocarbons; benzo(a)pyrene, 3-methylcholanthrene and 7,12-dimethyl-benz(a)anthracene destroy primordial oocytes in the rodent ovary in a time, dose and metabolism dependent manner. The goal of this research is to explore the relationship between ovarian metabolism of xenobiotic compounds and effects on reproductive performance.

PHS-6040
(Rev. 10-76)

Objectives

Reproduction is a complex interdependent process beginning with gamete release, continuing through fertilization, implantation, embryonic development, birth, growth and development. The reproductive process may be susceptable to interruption by xenobiotic compounds at various levels. Biologically foreign compounds may reversibly alter reproductive competence by interference with the regulatory mechanisms of the hypothalamic-pituitary-gonadal axis. Such reversible interactions would be expected to be self-limited and without consequence to the informational function of the gamete genome. Xenobiotic compounds which interrupt reproduction by direct action on the gamete however, may have profound effects on reproductive competence as well as the information transfer process inherent in gamete release and fertilization.

Several classes of xenobiotic compounds have been demonstrated to destroy oocytes and follicles in rodents. As the mechanism of action of these compounds in oocyte and follicle destruction is not understood the susceptability of other species, including humans, to similar effects by xenobiotic compounds is not known. In order to develop a better understanding of the effect of xenobiotic compounds on reproduction we are exploring in detail the mechanism of oocyte and follicle toxicity by the polycyclic aromatic hydrocarbons (PAH); benzy(a)pyrene (BP), 3-methylcholanthrene (MC), and 7,12-dimethylbenz(a)-anthracene (DMBA). Parallel investigations are also being carried out with other xenobiotic ovotoxic compounds, especially antitumor agents in collaboration with Dr. Snorri Thorgeirsson, LCHP, NCI (Z01 CM 06137-02 LCHP).

Major Findings

Using inbred strains of mice and rats we have investigated the time, dose and metabolism dependence of oocyte and follicle destruction. Initial studies utilizing inbred DBA/2N (D2) and C57BL/6N (B6) mice demonstrated differences in response of the ovarian microsomal P-450 dependent monooxygenases to PAH treatment, D2 mice treated with BP had no change in ovarian aryl hydrocarbon hydroxylase(AHH,benzo(a)pyrene 3 hydroxylase, EC 1.14.14.2) activity, but B6 mice treated with BP had a three fold increase in ovarian AHH activity. After treatment with the PAH; BP, MC, and DMBA primordial ooxytes were destroyed in both strains of mice in a time and dose dependent fashion. Oocyte destruction with all three compounds was more rapid in B6 than D2 mice and the ovotoxicity of the three PAH was BP < MC < DMBA. The competative inhibitor of PAH metabolism, α-naphthoflavone blocks oocxyte toxicity by the PAH, and the nonmutagenic PAH pyrene is not ovotoxic. These data suggest that oocyte destruction is specific and depended on metabolism. The specificity also extends to cell type. BP and MC seem to affect only the primordial oocytes with no demonstrable light or electron micrographic changes in the surrounding granulosa or ovarian stromal cells. DMBA however is much more toxic and destroys primordial oocytes and primary follicles as well as growing and large follicles.

Comparing ovarian AHH activity and oocyte toxicity in D2 and B6 mice suggested an association between inducible ovarian AHH activity and oocyte toxicity, to further explore this relationship we have investigated PAH oocyte toxicity as well as the metabolic profile of BP in Sprague-Dawley (SD) rats, a strain with PAH inducible ovarian AHH activity. SD rats are quite resistant

to PAH oocyte toxicity, suggesting that the hypothesized relationship between the BP 3-hydroxylase and toxicity was incorrect. Using high pressure liquid chromatography techniques many BP metabolites from ovarian metabolism were isolated and identified. These experiments demonstrated that the mouse ovary can metabolize BP in the 7,8,9,10 region much more rapidly (about 5 fold) than the SD rat. Although these steps of metabolic activation (involving oxygenation, hydration and oxygenation) are only the beginning steps in the ultimate development of toxicity they suggest that the increased toxicity observed in the mouse is the result of more rapid generation of the 7,8-dihydrodiol-9,10-oxide of BP, the compound thought to be the proximate mutagen.

Colaborative studies with Dr. Snorri Thorgeirsson LCMP, NCI have demonstrated primordial oocyte destruction by several antitumor drugs. The mechanism of action of these compounds is being explored.

Significance to Biomedical Research and Program of the Institute

Understanding the mechanisms of successful reproduction and the ways in which reproduction can be interrupted by xenobiotic compounds is of major importance. The model being explored, ovarian metabolism of PAH and oocyte destruction offers the possability of detailed exploration of the mechanism of action and effects of PAH on all aspects of ovarian function as well as reproduction.

Proposed Course

Further research is planned to explore the relationship between ovarian metabolism and oocyte toxicity in several other inbred strains of mice as well as other species. A more complete understanding of the pathways for toxication as well as detoxication will also be necessary as oocyte toxicity is the end point of a complex cellular process. The interaction of xenobiotic compounds and endogenous substrates (steroid hormones) for the microsomal P450 dependent monooxygenases will also be explored. The effects of xenobiotic compounds on the total reproductive process will also be assesed.

Publications

1. Gulyas, B.J. and Mattison, D.R.: Degeneration of mouse oocytes in response to polycyclic aromatic hydrocarbons. Anat. Record 193:863-882, 1979.

2. Mattison, D.R.: Oocyte destruction by xenobiotic compounds. Contemporary OB/GYN. In press.

3. Mattison, D.R. and Thorgeirsson, S.S.: Ovarian aryl hydrocarbon hydroxylase activity and primordial oocyte toxicity of polycyclic aromatic hydrocarbons in mice. Cancer Res. In press.

4. Mattison, D.R., West, D.W., and Menard, R.H.: Differences in benzo(a)pyrene metabolic profile in rat and mouse ovary. Biochem Pharm. In press.

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Endocrinology and Reproduction Research Branch

Project Numbers	Project Title	Principal Investigator
Z01 HD 00021-03	Bioassayable and Radioimmunoassayable ACTH	C. Strott
Z01 HD 00022-06	Renin-Angiotensin System and Aldosterone Regulation	K. Catt
Z01 HD 00035-07	The Structure and Function of Biologically Active Molecules	E. Gross
Z01 HD 00040-04	Experimental and Theoretical Studies of Hormone Receptor Interaction and Response Coupling	D. Rodbard
Z01 HD 00041-04	Cardiovascular Effects of Thyroid, Hormones, Catecholamines and Cardiotoxic Drugs	D. Rodbard
Z01 HD 00042-04	Mathematical and Statistical Theory of Gel Electrophoresis and Gel Filtration	D. Rodbard
Z01 HD 00043-04	Mathematics and Statistics of Radioligand Assays	D. Rodbard
Z01 HD 00146-04	Structure and Function of Chorionic Gonadotropins	H. Chen
Z01 HD 00147-04	Mechanism of Action of Gonadotropins and of ACTH	M. Dufau
Z01 HD 00148-04	Ontogeny of Gonadotropin Receptors and Leydig Cell Function	K. Catt
Z01 HD 00149-04	Bioassay of Serum Luteinizing Hormone (LH) and Chorionic Gonadotropin	M. Dufau
Z01 HD 00150-04	Characterization and Purification of LH/hCG Receptors and Adenylate Cyclase	M. Dufau
Z01 HD 00151-04	Regulation of Gonadotropin Receptors in Testis and Ovary	K. Catt
Z01 HD 00152-04	Characterization and Radioligand Assay of Gonadotropin Receptors	K. Catt
Z01 HD 00157-04	Physiology of Puberty	L. Loriaux
Z01 HD 00158-04	Pathophysiology of Gynecomastia and Hirsutism	L. Loriaux
Z01 HD 00160-04	Biology of the Adrenocortical Cell: Molecular Events in Steroid Biosynthesis and Secretion	C. Strott
Z01 HD 00161-04	Role of Sex Steroids in Regulation of FSH & LH Levels in Man	R. Sherins
Z01 HD 00162-04	Studies of a Specific FSH Inhibitor from Seminiferous Tubules	R. Sherins
Z01 HD 00163-04	Clinical Studies of Male Reproductive Disorders	R. Sherins
Z01 HD 00164-03	Strategy and Mechanisms of Fractionation	N. Nguyen
Z01 HD 00165-04	Isolation and Characterization of Protein Hormones & Other Active Proteins	A. Chrambach
Z01 HD 00170-03	A Randomized Study for the Treatment of Chromophobe Adenomas of the Pituitary Gland with Irradiation	C. Strott
Z01 HD 00171-03	Other PAGE Instrumentation and Procedures	A. Chrambach
Z01 HD 00173-03	Gel Electrofocusing Methods	N. Nguyen

Project Numbers	Project Title	Principal Investigator
Z01 HD 00174-03	Hormonal Control of Ovarian Proteoglycan Synthesis	D. Rodbard
Z01 HD 00175-02	Gel Elecrophoresis with Continuous Optical Scanning	B. Chen
Z01 HD 00176-02	Clinical Evaluation of Adrenocortical Hyper-Function	C. Strott
Z01 HD 00179-01	Inferior Petrosal Sinus Catherization in Diagnosis and Treatment of Pituitary Tumors	C. Strott
Z01 HD 00180-01	Biology of Hormone Binding Proteins	B. Nisula
Z01 HD 00181-01	Steroid Antagonists	L. Loriaux
Z01 HD 00182-01	Structure, Function, and Physiology of Glyco-protein Hormones	B. Nisula
Z01 HD 00183-01	Catechol Estrogens: Physiological Effects	L. Loriaux
Z01 HD 00184-01	Regulation of Pituitary Hormone Secretion	K. Catt

ANNUAL REPORT
of the
Endocrinology and Reproduction Research Branch
National Institute of Child Health and Human Development
October 1, 1978 through September 30, 1979

SUMMARY

The Endocrinology and Reproduction Research Branch maintains research programs in the basic and clinical aspects of endocrine systems and the control of reproductive processes. The Branch provides training for Clinical and Research Associates, and Visiting Fellows, in laboratory and clinical investigation. This includes a combined endocrinology training program with NIAMDD for clinical associates, and a fellowship program in reproductive endocrinology with the Armed Forces Institute.

The research performed in the current year has extended earlier work on the analysis and characterization of protein molecules and hormone receptors, the structure-function relationships of biologically active molecules, the regulation of hormone synthesis and secretion, and the analysis of mechanisms of hormone action. Further work was performed upon the investigation of pituitary gonadal relationships, ovarian function during the menstrual cycle and pregnancy, and several areas of clinical investigation. This report summarizes the progress made in methods for the analysis and characterization of peptide hormones, their receptor sites and cellular actions, the biochemical regulation of steroid hormone synthesis and secretion, and the role of steroid hormones in cellular processes and clinical disorders.

The Section on Biophysical Endocrinology performs mathematical and statistical analysis of endocrine regulatory processes and the physical properties of protein hormones studied by polyacrylamide gel electrophoresis; applies mathematical modeling, computer simulation and biophysical methods to studies on hormone receptor interactions and regulation of the ovarian cycle; and investigates cardiovascular changes in thyroid disease. Current research includes the development of improved methods for analysis of hormone-receptor systems, including computerized methods for characterization of binding isotherms in terms of a continuous distribution of affinities; for analysis of the limiting slopes of Scatchard plots in terms of the moments of the affinity distribution; and for simulation and curve-fitting of systems involving multiple ligands reacting simultaneously with numerous discrete classes of receptors. The latter method permits combination of data from several experiments, with adjustment for receptor concentration and non-specific binding between experiments, and appropriate weighting. These programs have been employed to analyze LHRH receptor binding in pituitary tissue, and to analyze opiate binding to brain.

Experimental studies have demonstrated that the benzodiazepine receptor is coupled to a chloride anion channel, and that the influence of GABA or diazepam binding may be mediated through this channel. Other studies include the effects of experimental hyperthyroidism, ACTH treatment, and nucleotides upon the benzodiazepine receptor. Studies were also performed on the chemical composition, structure and physiology of proteoglycans in ovarian follicular fluid.

The Section on Developmental Endocrinology performs research on the role of endocrine factors in biological differentiation of human and animal tissues, to analyze the endocrine control of fetal, pubertal and senescent differentiation. This includes studies on hypothalamic-pituitary-gonadal interrelationships during growth and development, the role of sex steroids in skeletal maturation, and of steroids in growth and development; and the interaction between Leydig cells and seminiferous tubule function. Current research on the physiology of adrenarche has shown that only man and certain primates (chimpanzee, mountain gorilla) exhibit an adrenarche characterized by increased production of dehydroepiandrosterone and androstenedione. This process was found to be independent of gonadarche, and the pattern of recovery from adrenal suppression was found to differ for cortisol and the adrenal androgens. Patients with "delayed" adrenarche were identified, and found to have hypothalamic and/or pituitary lesions. Experimental studies on adrenarche were initiated in the chimpanzee, employing human pituitary extract in hypophysectomized animals, and in the dog, in which histologic correlates of adrenarche were identified. The accompanying increase in plasma 17 hydroxy-progesterone, and the almost complete absence of 17-20 desmolase in the dog suggests that this species may provide a useful model for the general process of adrenarche. In other studies, seeking a glucocorticoid antagonist with in vivo activity, delta-1, (9, 11) deoxycortisol was found to be an effective compound, presumably due to resistance to 11-hydroxylation. Additional compounds with resistance to 11-hydroxylation were designed and will be synthesized and tested for biological activity.

Measurements of glycoprotein hormones and their metabolites in biological fluids were further improved to provide improved sensitivity and accuracy for hormone assay. Highly sensitive radioimmunoassays for TSH and urinary hCG were developed, and the distribution and metabolism of hCG sub-units were analyzed in man. The mechanism of hyperthyroidism in patients with choriocarcinoma was further studied by analysis of thyroid receptor binding activity and other methods to characterize the thyrotropic factor in such patients.

Clinical studies of male reproduction were continued in normal subjects and patients with testicular dysfunction. In relation to the recognition that only five to ten million sperm are required for impregnation, metabolic studies on normal and immotile sperm were conducted. Immotile sperm from infertile men did not utilize fructose as an energy source, showed increased lipid peroxidation, and exhibited no protein carboxyl methylase activity, the latter enzyme being required for ciliary movement. These findings will be further applied to the evaluation of male reproductive potential, and disorders of fertility.

Regulation of gonadotropin secretion by sex steroids was further explored in man during studies with the aromatase inhibitor delta^{1} testolactone and the anti-estrogen, clomiphene. These studies established a major role for estradiol in the regulation of gonadotropin secretion in men. The role of sex steroids in LH and FSH regulation was also analyzed in the rat, in which physiologic doses of testosterone were shown to maintain normal gonadotropin levels after castration. A monotropic increase in FSH occurred when androgen deficiency was associated with a relative increase in plasma estradiol. These findings indicate that gonadal steroids serve as the primary regulators of LH and FSH secretion, and that the existence of a non-sex steroid inhibitor of FSH secretion (inhibin) need not be postulated.

The Section on Hormonal Regulation performs research on the regulatory aspects of peptide hormone action at the target cell level. This includes analysis of the factors involved in hormone-receptor interaction and stimulus-response coupling, during research on the control of gonadal steroidogenesis by gonadotropins, and of aldosterone secretion by angiotensin II and other hormones.

Current research on gonadotropin action has focused on the receptors for luteinizing hormone (LH) in animal and human gonads. LH receptors were found to be regulated by the prevailing concentration of circulating LH, and the effects of receptor loss upon gonadal target cell function were analyzed in the testis and ovary. In both tissues, loss of receptors was preceded by an acute desensitization phase in which plasma membrane responses to the hormone were abolished, followed by a longer-term refractory phase in which receptor loss contributed to the inability of hormone to evoke target cell responses in vitro. In the testis, defects in the steroidogenic pathway were also detected, during the phase of impaired androgen production in the desensitized gonad. The desensitizing effects of gonadotropin releasing hormone (GnRH) were also investigated in the testis, which exhibited marked loss of both LH and prolactin receptors following treatment with the natural sequence releasing hormone, with impairment of Leydig cell responses to hormone stimulation. The more profound degree of testis regression produced by GnRH treatment suggested a direct action of the peptide at the gonadal level, as well as its down-regulating effects via release of endogenous hormones.

The LH receptors of the human and rhesus monkey testis were found to exhibit high specificity for human hormones, with relative insensitivity to animal gonadotropins. This contrasts with the broad species-reactivity of the rat testis LH receptors, and explains the inability of animal gonadotropins to stimulate responses in the primate testis. In other studies, the action of GnRH upon LH release was analyzed in cultured rat pituicytes, and was found to be independent of cyclic AMP production and action. Binding studies on pituitary receptors for releasing hormone were initiated with a labeled GnRH analogue, which identifies the high affinity receptor sites and permits studies on the structural requirements for binding to the GnRH receptors.

The actions of angiotensin II were investigated in the adrenal zona glomerulosa, where receptors for the octapeptide form the locus at which the renin-angiotensin system controls aldosterone secretion during alterations in sodium balance. The adrenal receptors were functionally similar to those present in smooth muscle, and similar sites were identified in the anterior pituitary glands. In the adrenal, the role of angiotensin II was further analyzed in altered sodium imbalance, providing conclusive evidence for the obligatory function of angiotensin II in the adrenal response to sodium deficiency. Thus, blockade of angiotensin II formation by administration of converting-enzyme inhibitor during the onset of sodium deficiency completely abolished the changes in angiotensin II receptors and aldosterone responses that accompany decreased sodium intake. Thus, angiotensin II serves to increase its own receptors in the adrenal gland, and to sensitize the zona glomerulosa cells to the action of circulating angiotensin II during sodium deficiency. The

actions of angiotensin II on target cell responses were analyzed in adrenal glomerulosa cells, and in dispersed smooth muscle cells. In the adrenal, stimulation of aldosterone production by angiotensin II was independent of cyclic AMP formation, suggesting the existence of an alternative mechanism for the steroidogenic action of the octapeptide. The effect of angiotensin II on aldosterone production is highly calcium dependent, suggesting that calcium may serve as the second messenger for the actions of angiotensin II in the adrenal.

The Section on Molecular Endocrinology investigates the molecular basis of peptide hormone action, with particular emphasis on the control of gonadal and adrenal cortical function. This includes analysis of the nature and composition of gonadotropin receptors, and the activation of steroid biosynthetic pathways in the gonads and adrenal. Investigations are also performed on the properties and biological activity of circulating gonadotropins, during physiological states and in clinical disorders of pituitary and gonadal function. In recent studies, the activation of protein kinase and steroid responses in adrenal, testis and ovarian target cells by the respective trophic hormones was shown to depend on minute changes in intracellular cyclic AMP levels. This was made possible by the development of techniques for measurement of cyclic AMP bound to the protein kinase receptor protein, which permitted demonstration of nucleotide binding to regulatory sub-units during stimulation of target cells by physiological concentrations of protein hormones. In the adrenal, the activation process was shown to be solely attributable to cyclic AMP formation, and the proposed role of cyclic GMP as a second messenger in this tissue was excluded. In the testis, the predominant 6.2 S protein kinase holoenzyme was activated during hormone stimulation, and the minor 4 S form of the enzyme was dissociated only at high hormone concentrations, suggesting differential activation of the enzymes during hormone action. In the adrenal fasciculata, two forms of cyclic AMP-dependent protein kinase were identified with MWs of 160,000 and 100,000. The action of ACTH on steroidogenesis was mediated by activation of both protein kinase holoenzymes by intracellular cyclic AMP.

In related studies, the role of trophic hormones and steroid metabolites in the maintenance of steroidogenic enzymes was investigated. A partial defect in steroidogenesis was characterized in rat Leydig cells from animals desensitized by treatment with gonadotropin or GnRH, which resulted in a lesion of the 17-20 desmolase enzyme. This was shown to be attributable to increased estradiol production by studies employing estrogen antagonists and aromatase inhibitors, which prevented the impairment of testosterone responses caused by gonadotropins or GnRH.

The recently-developed high in vitro bioassay for circulating LH was further refined for application to studies in the rat. Analysis of the low levels of bioactive and immunoreactive LH in male and female rats was performed by modified bioassay and radioimmunoassay procedures with improved sensitivity and precision. Changes in LH bioactivity were observed at the time of the LH surge at estrous, and following castration, and androgens or their metabolites were shown to modify the bioactivity of circulating and stored forms of the LH molecule.

Further analysis of the physical and functional relationships of LH receptors and adenylate cyclase in gonadal membranes was performed by transfer

of receptors from ovarian extracts to heterologous steroidogenic cells, taken from the adrenal gland. By this approach, ovarian LH receptors were incorporated into adrenal cells and shown to be functionally coupled to adenylate cyclase and steroidogenesis. These and earlier studies indicate that while gonadotropin receptors and adenylate cyclase exist in the cell membrane as separate entities, the two species become associated to perform an active complex during receptor occupancy by the homologous hormone. In related studies, gonadotropin receptors were extracted from testis and ovary under conditions providing water-soluble LH sites with high specificity for gonadotropins. Such receptors exhibit a range of MWs and are stable to lyophilization. The specific binding site for LH is much smaller than the 6.5 S receptor extracted by detergents, and the stability of the water-soluble LH receptors will permit further purification and analysis of the structural determinants of gonadotropin binding.

The Section on Molecular Structure performs research on the structural and functional properties of biologically active peptides. This includes the elucidation of rare structural features in amino acids and peptides, development of new techniques for peptide analysis and synthesis, and synthesis of peptides for biological and clinical investigations.

Analytic studies were performed on the rare amino acids, hydroxyaspartic acid and dehydroxyaspartic acid. Hydroxyaspartic acid is present in the heterodepic multicyclic peptides CINNAMYCIN and DURAMYCIN, and dehydroaspartic acid is formed from hydroxyaspartic acid under relatively mild acidic conditions. The generation of dehydroaspartic acid is of physiological significance in regulatory mechanisms involving the addition of nucleophiles to double bonds. In osteogenesis imperfecta, hydroxylysine and glycosides of this amino acid were separated by ion exchange chromatography and quantitated, providing an approach to the diagnosis of this disease.

Synthetic studies include the preparation of (a) luteinizing hormone releasing hormone (LHRH), synthesized by various techniques for clinical studies directed at the induction of ovulation; (b) analogs of the linear gramicidins, synthesized and investigated for their ion transport properties, including a pentadecapeptide capable of forming transmembrane channels and transporting alkali ions across lipid bilayers. (c) The gramicidin analogs with alternating L- and D-valine and shorter fragments of the same was prepared for studies on lipid bilayers (ion transport phenomena), and their potential to affect neonatal and neoplastic tissue.

Synthesis of heterodetic multicyclic peptides (e.g., Nisin) was performed by the stepwise condensation of Rings A, B, C. Initial requirements of the synthesis were met by providing access to the unique structural feature seen in Ring A of nisin: the alpha, beta-unsaturated amino acid dehydroalanine in position 5, and the presence of meso-LANTHIONINE (position 3 through 7). The former amino acid incorporated in the appropriate peptide structure is accessible via beta-elimination from precursors with O- and/or S-substituted serine and cysteine, respectively, the latter via the decomposition of sulfonium salts obtained from derivatives of cysteine and beta-halo alanine.

The Adrenal Research Unit investigates the basic and clinical aspects of adrenal steroid-secreting cells. Current research is aimed at the identification

and characterization of non-catalytic adrenal proteins that interact with specific steroid ligands. Also, the role of microtubules and contractile proteins in steroid synthesis is under investigation, together with the characterization of adrenocortical secretory proteins. Binding proteins for cholesterol and cholesterol sulfate have been characterized, and comparable proteins which bind pregnenolone and pregnenolone sulfate have been identified. These binding proteins for steroid precursor models are being purified, and will be employed to reconstitute cholesterol lyase and other steroid mixed-function oxidase systems responsible for steroid hormone biosynthesis. In related studies, the role of cellular compartmentalization, and the possible involvement of microtubules and microfilaments, was examined in steroidogenic cells. Tubulin was identified in the adrenal cortex, and anti-microtubular drugs were shown to stimulate steroid synthesis to the levels evoked by ACTH or cyclic AMP. A comparison of neural (brain) and non-neural (adrenal) microtubules was performed after purification of tubulin from both tissues. The role of microtubules in the regulation of adrenocortical steroidogenesis will be further studied in cultured adrenal cells, employing correlations between morphological and biochemical procedures for identification of microtubules and microfilaments.

The Electrophoresis Unit performs research on techniques and applications of highly resolving electrophoretic fractionation systems. In recent studies, preparative procedures for protein isolation in high-yield and purity were further developed, and can be applied to protein loads in the 10-100 mg range. Technical developments include methods to measure conductance gaps during isoelectric focusing, and to determine the percent conversion of acrylamide to polyacrylamide during the cross-linking polymerization reaction. Applications of these fractionation procedures to biological systems include the analysis of solubilized adenylate cyclase and cytochrome P-450, and membrane receptors for insulin. Other studies included the analysis of protein heterogeneity due to radioiodination (of tetanus toxin and human growth hormone), the separation of two major isohormones of human prolactin, the characterization of the glucocorticoid receptor of the chick embryo, and the analysis by isoelectric focusing of human chorionic gonadotropin. In the latter study, biological activity and high sialic acid content were found to be associated, indicating the importance of sialic acid for the *in vivo* biological effects of this glycoprotein hormone.

The Protein Chemistry Unit performs research on the structure and biological properties of gonadotropic hormones. The hCG-like substance previously identified in human pituitary fractions was further characterized and shown to possess all the properties of hCG. This material will be isolated on a larger scale and subjected to more detailed physico-chemical analysis. Antisera raised against the C-terminal peptide fragments of the hCG beta sub-unit showed specificity for hCG and lack of recognition of human luteinizing hormone during radioimmunoassay. Immunoabsorbents prepared from such antisera have been employed to concentrate hCG and hCG-like substances in the presence of hLH, permitting increased sensitivity of measurements for hCG in biological fluids. The purification of chimpanzee chorionic gonadotropin was performed by group-specific affinity chromatography and ion exchange chromatography, yielding a product with relatively high biological activity which will be analyzed for structural homologies with the sub-units and C-terminal beta sub-unit portion of hCG. This group of studies will provide information about the relationships between hCG and hLH, and the specific biological, chemical and immunological functions of the unique portion of the beta sub-unit of the hCG molecule.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00021-03 RR

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)
Bioassayable and Radioimmunoassayable ACTH

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	Charles A. Strott	Senior Investigator	ERRB, NICHD
	Kevin Catt	Chief	ERRB, NICHD

COOPERATING UNITS (if any)

None

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Two independent methods of characterizing and quantitating ACTH from clinical samples are being developed, a classical radioimmunoassay with materials supplied by the NIAMDD and a bioassay, using as an endpoint the corticosterone production of isolated rat adrenal fasciculata cells. Both assays have been found to be sensitive enough for human use and to have specificity which is suitable. An extraction procedure is being developed and is the final step in completing both assays.

In addition gel chromatography and ion exchange chromatography are being used to characterize the ACTH. Also, the columns are being used to prepare biologically active ^{125}I-labelled ACTH for receptor studies.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00022-06 RR

PERIOD COVERED

October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Renin-Angiotensin System and Aldosterone Regulation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	K. J. Catt	Chief	ERRB, NICHD
Other	G. Aguilera	Visiting Scientist	ERRB, NICHD
	J. Fakunding	Staff Fellow	ERRB, NICHD
	K. Fujita	Visiting Fellow	ERRB, NICHD
	A. Capponi	Fogarty International Fellow	ERRB, NICHD
	A. Schirar	Guest Worker (French Government Fellow)	ERRB, NICHD

COOPERATING UNITS (if any)

None

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Hormonal Regulation

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.0	5.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This program is directed at the elucidation of the role of the renin-angiotensin system and other factors in the regulation of aldosterone secretion by the zona glomerulosa of the adrenal gland. Specific areas of research include the characterization and regulation of angiotensin II receptors, the actions of angiotensin II and other regulators of aldosterone secretion, and the production and metabolism of angiotensin II. Major emphasis has been placed upon the use of enzyme-dispersed adrenal cells for analysis of the relations between angiotensin II receptors and aldosterone production, and the interactions between angiotensin II and other factors such as ACTH, sodium and potassium levels. The effects of chronic electrolyte imbalance on adrenal receptors and steroid responses are studied, to analyze the acute effects of sodium balance on the concentration and affinity of angiotensin II receptors in adrenal and uterine smooth muscle. These studies are providing insights about the control of adrenal and vascular sensitivity to angiotensin II during sodium deficiency, the mechanisms by which angiotensin II regulates the concentration of its receptors in adrenal and smooth muscle, and the activation mechanisms that are initiated by occupancy of angiotensin II receptors.

PHS-6040
(Rev. 10-76)

Objectives:

The objectives of this project are to clarify the role of the renin-angiotensin system in the regulation of aldosterone secretion in physiological and pathological conditions. This includes characterization of the properties and regulation of angiotensin II receptors, and their role in the activation of adrenal steroidogenesis; elucidation of the mechanisms of action of angiotensin II and other regulators of aldosterone secretion in the zona glomerulosa; comparison of the angiotensin II receptors of adrenal and smooth muscle, and their regulation by electrolyte changes and angiotensin II; and evaluation of the role of the renin-angiotensin system and aldosterone in certain forms of clinical hypertension.

Methods Employed:

Radioimmunoassays of renin activity, angiotensin II, aldosterone, corticosterone, cortisol, and cyclic nucleotides. Binding studies with ^{125}I- and ^{3}H-angiotensin II in adrenal cells and membranes, and uterine or aortic membranes, enzymatic dispersion of adrenal glomerulosa and fasciculata cells. In vivo and in vitro stimulation of aldosterone responses by rat and dog adrenal cells. Measurement of agonist and antagonist activities of angiotensin II fragments and analogues upon receptor binding and aldosterone production in vitro.

Major Findings:

1. Properties of angiotensin II receptors.

The structural requirements for binding of angiotensin II to adrenal and uterine receptors and for activation of adrenal steroidogenesis, were studied in canine tissues. The ability of analogues and COOH-terminal and NH_2-terminal fragments of angiotensin II to inhibit binding of ^{125}I-angiotensin II was analyzed in dog adrenal and uterine particles. In both tissues, COOH-terminal peptides displayed a progressive fall in binding inhibition activity with decreasing chain length and the (5-8)-tetrapeptide was the shortest active fragment. Of the NH_2-terminal fragments, only the des-Phe^{8}-heptapeptide retained the ability to inhibit the binding of ^{125}I-angiotensin II to adrenal and uterine receptors. A close correlation was found between the equilibrium dissociation constants for a series of angiotensin II peptides in adrenal cortex and uterine particles. The hypothalamic peptide, luteinizing hormone-releasing hormone, and some of its analogues also inhibited ^{125}I-angiotensin II binding in adrenal and uterus, an effect attributable to structural similarities with the COOH-terminal sequence of angiotensin II. When the binding properties of the angiotensin analogues were compared with their aldosterone-stimulating activities in isolated adrenal glomerulosa cells, the COOH-terminal (4-8)-pentapeptide was the shortest active fragment, the NH_2-terminal (1-7)-heptapeptide behaved as a weak antagonist, and the shorter tetra- and tripeptides were inactive. A close correlation was observed between the dissociation constants and the activation constants of the peptides that stimulated aldosterone production. Although luteinizing hormone-releasing hormone was inactive on steroidogenesis,

des-pGlu^{1}-luteinizing hormone-releasing hormone displayed partial agonist activity upon aldosterone production in vitro.

These studies show that dog adrenal cortex and uterine receptors for angiotensin II are functionally identical in terms of binding affinity for several angiotensin II analogues. It is also apparent that the integrity of the COOH-terminus is required for receptor binding and induction of the biological response, which is retained by peptides as short as the COOH-terminal (4-8)-pentapeptide of angiotensin II.

2. Regulation of adrenal sensitivity.

(a) Direct actions of angiotensin II on adrenal receptors.

During sodium deficiency, adrenocortical sensitivity to angiotensin II is increased in several special species. Following acute sodium restriction in the rat, adrenal receptor sites for angiotensin II show increased concentration and binding affinity, and aldosterone responses to angiotensin II are increased both in vivo and in vitro. Since circulating renin and angiotensin II levels are elevated during sodium deficiency, these changes in the adrenal gland could be mediated by the trophic action of angiotensin II on the zona glomerulosa. To determine whether angiotensin II modulates its own receptors as well as the aldosterone secretory response, angiotensin II receptors and aldosterone production in the zona glomerulosa of rats were analyzed after elevation of circulating angiotensin II levels by minipump infusion. These studies provided evidence that angiotensin II can regulate its own receptor sites as well as steroidogenic responsiveness in the adrenal glomerulosa cell.

(b) Sodium intake and adrenal sensitivity.

The relationships between plasma renin activity (PRA) and plasma aldosterone were evaluated in rats maintained on altered dietary sodium or potassium intake for 6 weeks. A bivariate method of analysis was utilized to determine the correlations between these two dependent variables in the presence and absence of anesthesia for blood collection. Sodium restriction had a direct stimulatory effect on both renin and aldosterone, and a high sodium diet had the opposite effect. A significant positive correlation was observed between PRA and plasma aldosterone with variations in dietary sodium intake, either in the presence or absence of anesthesia for blood collection. With variations in dietary sodium intake, linear changes in PRA were accompanied by logarithmic changes in plasma aldosterone.

Varying potassium intake caused a negative correlation between PRA and aldosterone. High potassium intake was accompanied by significant suppression of PRA and marked stimulation of aldosterone production, to a degree that exceeded low sodium intake. In contrast, low potassium intake was associated with a 7-fold increase in PRA, with no increment in aldosterone production.

The influence of dietary maneuvers on renin and aldosterone was not obscured by anesthesia. However, anesthesia had a more pronounced stimulatory

effect on renin than on aldosterone secretion. These findings demonstrate that variations in either sodium or potassium intake in the rat influence adrenal sensitivity to the renin-angiotensin system.

3. Mechanisms of angiotensin II action on steroidogenesis.

(a) Role of cyclic nucleotides and ATPase.

Dog and rat adrenal glomerulosa cells and subcellular fractions were utilized to evaluate the mechanism of angiotensin II- and angiotensin III-induced aldosterone production. The effects of angiotensin, ACTH, and potassium were compared on cyclic AMP and cyclic GMP in isolated glomerulosa cells and adenylate cyclase activity in subcellular fractions. The effect of angiotensin II was also assessed on Na^+-K^+-activated ATPase of plasma membrane enriched fractions of dog and rat adrenals. These studies demonstrated no effect of angiotensin II or angiotensin III on either adenylate cyclase, cyclic AMP, cyclic GMP, or Na^+-K^+-dependent ATPase activity over a wide range of concentrations. Potassium ion in concentrations that stimulate significant aldosterone production were also without effect. The negative effects of angiotensin and potassium were contrasted against a positive correlation between an ACTH-induced effect on aldosterone production, adenylate cyclase, and cyclic AMP accumulation.

These studies have demonstrated that neither adenylate cyclase, cyclic AMP, cyclic GMP, or Na^+-K^+-activated ATPase seem to be directly involved in the mechanism of action of angiotensin on aldosterone production in the rat and dog adrenal glomerulosa.

(b) Role of calcium in angiotensin II action.

Analysis of the role of calcium in the regulation of aldosterone production showed that the steroidogenic responses of isolated adrenal cells to ACTH, angiotensin II, and potassium were highly dependent on the extracellular calcium concentration. Physiological concentrations of calcium were required for maximal aldosterone responses to all three regulators, and steroidogenesis was progressively reduced by decreasing calcium concentrations. ACTH stimulated aldosterone and cAMP responses at all calcium concentrations tested, and the production of steroid was correlated with the increase in cAMP formation. Reduction of extracellular calcium concentration caused an increase in the ACTH concentration required for half-maximal steroid and cAMP production and a decline in the maximal output of aldosterone. When zona glomerulosa cells were stimulated by exogenous cAMP, cholera toxin, or serotonin, decreased calcium concentration caused a decline in the amount of steroid produced but did not change the agonist concentration required for half-maximal stimulation. These findings suggest that calcium is required for the coupling of ACTH-receptor complexes with adenylate cyclase in the glomerulosa cell membrane and also at an intracellular locus subsequent to the action of cAMP. In contrast, angiotensin II and potassium did not increase cAMP production at any of the calcium concentrations tested, but both regulators stimulated aldosterone production in a calcium-dependent manner. Reduction of extracellular calcium concentration

caused a decrease in the amount of steroid produced but did not change the concentration of either angiotensin II or potassium required for half-maximal stimulation of aldosterone production. Binding of angiotensin II to glomerulosa cell receptors was not altered in the absence of calcium, again indicating the postreceptor locus of a calcium-dependent step in the steroidogenic pathway. Although calcium is required for the steroidogenic response of zona glomerulosa cells to ACTH, angiotensin II, and potassium, the calcium requirement of ACTH action is quantitatively distinct from that for angiotensin II and potassium, consistent with a more calcium-dependent mechanism of action of the latter regulators.

4. Regulatory sites for the control of aldosterone secretion.

The effects of angiotensin II, ACTH, and potassium on early and late steps of aldosterone biosynthesis were studied in collagenase-dispersed cells from rat adrenal capsules and dog zona glomerulosa. Isolated cells were incubated with a cyanoketone derivative to isolate the early and late portions of the biosynthetic pathway. In this way, the formation of pregnenolone and later steps of biosynthetic conversion in the absence of endogenous precursors could be measured independently during stimulation by the three regulators. During incubation for 3 h with 10 nM angiotensin II, 10 nM ACTH, or 15 mM potassium, marked stimulation of pregnenolone production was observed in glomerulosa cells of both species. In rat glomerulosa cells, each of the three regulators also stimulated conversion of exogenous corticosterone to aldosterone. In dog glomerulosa cells, angiotensin II and potassium, but not ACTH, stimulated the conversion of corticosterone to aldosterone.

In rat glomerulosa cells, aldosterone production from exogenous progesterone and deoxycorticosterone was 70-100% higher than from corticosterone, whereas dog cells showed equal conversion from all three precursors. Incubation with aminoglutethimide decreased conversion from corticosterone to aldosterone in both species, and from deoxycorticosterone to aldosterone in dog cells. However, aminoglutethimide did not reduce conversion of DOC to aldosterone in rat glomerulosa cells. These results demonstrate that two of the major physiological regulators of aldosterone production (angiotensin II and potassium) stimulate both early and late steps of steroid biosynthesis in rat and dog adrenal cells. ACTH also stimulates at both sites in rat capsular cells, but acts only on the early step in dog glomerulosa cells. Furthermore, the lack of an inhibitory effect of aminoglutethimide on conversion of deoxycorticosterone to aldosterone in rat adrenal cells reveals an alternate late pathway to aldosterone synthesis in this species.

5. Cellular metabolism of angiotensin II.

The steroidogenic activities and receptor-binding properties of angiotensin II and des-Asp^{1}-angiotensin II were analyzed and compared with the metabolism of each peptide during incubation with isolated rat and canine adrenal glomerulosa cells. In isolated rat glomerulosa cells, equimolar concentrations of each peptide stimulated aldosterone production similarly over

the first 20 min of incubation. However, at later times, the des-Asp^1 heptapeptide was consistently less active, with one-third the potency of the octapeptide at 40 min, and only one-tenth that of the octapeptide after 120 min. In dog glomerulosa cells, the des-Asp^1 heptapeptide was less rapidly inactivated and retained one-third the potency of the octapeptide after 120 min of incubation. In each species, the maximum aldosterone response to the two peptides was similar at all time intervals studied up to 120 min. Also, no additivity between maximal concentrations of angiotensin II and des-Asp^1-angiotensin II was observed. Assay of angiotensin II receptors with radioiodinated and tritiated peptides in rat glomerulosa cells and adrenal particles showed identical receptor concentration for each peptide, with somewhat lower affinity for the heptapeptide (K_a = 0.7 x $10^9 M^{-1}$ for the heptapeptide vs. 2.7 x $10^9 M^{-1}$ for angiotensin II).

The metabolism of angiotensin in isolated glomerulosa cells was analyzed by thin layer chromatography and assay of peptides in the incubation medium. Extensive degradation of angiotensin II during incubation with rat cells was detected, with relatively little conversion to the 2-8 and 3-8 peptides. RIA of medium peptides showed decreases to 50% and 30% for angiotensin II and des-Asp^1-angiotensin II at 40 min, and to 26% and 10% at 120 min, respectively. Similar results were derived with radioreceptor assay and bioassay by stimulation of aldosterone production in fresh glomerulosa cells. In contrast, metabolism of angiotensin during incubation with dog adrenal glomerulosa cells was less rapid, and both peptides were degraded at the same rate. Analysis of labeled peptides eluted from rat glomerulosa cells after *in vivo* or *in vitro* binding of [^{125}I]iodoangiotensin II showed that more than 90% of the bound radioactivity was composed of the octapeptide. These studies have revealed extensive metabolism of angiotensin peptides by isolated rat glomerulosa cells, with more rapid degradation of des-Asp^1-angiotensin II than of the native octapeptide. Such peptide metabolism contributes to the apparent discrepancy in biological potencies of angiotensin II and des-Asp^1-angiotensin II on aldosterone production in isolated rat glomerulosa cells. It is also evident that angiotensin II acts upon rat and dog glomerulosa cells to evoke aldosterone production without prior conversion to the heptapeptide.

Significance:

This project continues to provide new information about the control of aldosterone secretion, and the role of the renin-angiotensin system in adrenal regulation and smooth muscle function.

Proposed Course:

The role of calcium and ion shifts in angiotensin action, and the steroid biosynthetic changes induced by stimuli of aldosterone secretion, will be analyzed in more detail. The relations between changing sodium balance and angiotensin II receptors in adrenal and muscle will be further studied in the acutely sodium-restricted rat and dog. The role of both systemic and locally formed angiotensin II in adrenal regulation will be examined in rat and dog, employing

blockers of angiotensin formation and action during sodium restriction. Further characterization of angiotensin II receptors will be performed by affinity-labeling and solubilization of adrenal and uterine receptors for purification and physicochemical analysis.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00035-07 RR

PERIOD COVERED

October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Structure and Function of Biologically Active Molecules

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Endocrinology and Reproduction Research Branch

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5	1	4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Peptides with unique structural features display distinct biological properties. The studies of this project are directed at the analysis and synthesis of molecules with rarely encountered amino acids. Of foremost concern are: (a) the synthesis of precursors of ring A of nisin, a peptide with α,β-unsaturated and thioether amino acids; (b) the action of peptide structures based on nisin and/or the linear gramicidins on neonatal and neoplastic tissue; (c) the synthesis of analogs of the gramicidins A, B, and C, and the correlation of their structures with ion-transport across lipid bilayers; (d) the synthesis of peptides with agonistic and antagonistic effects on fertility regulating hormones inclusive of the large-scale preparation of the luteinizing hormone releasing factor (LRF) for clinical purposes; (e) the exploration of dehydroalanine resins for the synthesis of peptide amides; (f) the synthesis of analogs of opioid peptides with agonist and/or antagonist properties; (g) the synthesis of analogs of the pentapeptide L-Phe-D-Leu-L-Phe-D-Leu-L-Phe with chemotactic and anti-chemotactic characteristics; (h) the structural investigation of the heterodetic pentacyclic peptides CINNAMYCIN and DURAMYCIN; (i) the development of new techniques for peptide synthesis, amino acid analysis, and the nonenzymatic cleavage of the peptide bond.

Project Description

Objectives:

Structural eleucidation, chemical modification, and partial and/or total synthesis of biologically important peptides which: (a) incorporate unique structural features brought about by the presence of α,β-unsaturated and thioether amino acids; (b) represent portions of the sequence of hormones of the reproductive endocrine system and are useful for immunological investigations and applications; (c) display hormone releasing or hormone release inhibiting properties; (d) affect neonatal and neoplastic tissue; (e) show ionophoric characteristics and mediate the transport of ions across lipid bilayers and biological membranes; (f) are chemotactic or anti-chemotactic for neutrophils; (g) are structural variants of the naturally found amino acid assemblies with opiate like activities. Chemical, physical, and biological studies with membrane active peptides. Chemical modification of amino acids and development of nonproteolytic methods for the selective cleavage of peptide bonds. Isolation and characterization of subcellular constituents from developing tissue. Development of new approaches to the synthesis of peptides of physiological interest. Design, development, and implementation of new techniques and instrumentation in amino acid analysis and peptide synthesis. Development of amino acid analytical approaches for the detection of uncommon constituents in physiological fluids with the aim of providing a tool for the early diagnosis of pathological states.

Methods Employed:

Highly selective and thermostable proteases (e.g. thermolysin) in combination with newly developed nonproteolytic techniques for the structural elucidation of peptides with infrequently seen amino acids. Dehydroalanine linkage of amino acids to carriers in the solid phase synthesis of peptides and peptide amides. Alkylamidated residues of dehydroalanine to incorporate ω-amide functions in endo-positions of the peptide chain. Novel-type protecting groups meeting the requirement of highly selective removal in peptide synthesis. Techniques for the synthesis of amino acids with multiple centers of chirality under the conditions of stereochemical control. Advanced techniques for the separation of closely related molecular species and their analysis, e.g. specifically developed sample application and data acquisition in amino acid analysis; counter current distribution, conventional and high pressure liquid chromatography; electrophoresis; circular dichroism; polarimetry; mass spectrometry; nuclear magnetic resonance; X-ray analysis.

Major Findings:

Peptide Synthesis and Biology: (a) Leuteinizing Hormone Releasing Factor (LRF) synthesized by various techniques of peptide bond formation. The highly purified products meet all applicable chemical and physical criteria. Biologically, they are fully active and ready to be entered in clinical studies aimed at the induction of ovulation. The preparation of the decapeptide releasing factor in gram quantities necessitated the development and application of highly refined techniques of peptide synthesis to incorporate the guanido group of arginine and the terminal carboxamido function. Similarly, approaches of activation and protection had to be modified for the

incorporation of sensitive amino acid residues, such as tryptophan, tyrosine, and serine. Much experience has been gained with the improvements made in the approaches to the synthesis of a delicate peptide and bearing its fruit now that amino acids are being assembled to give molecular species that are hyperactive or display antaganistic properties. (b) Analogs of the Linear Gramicidins: i. HCO(L-Ala-Gly)$_7$-L-Ala-NHCH$_2$CH$_2$OH constitutes a distinct departure from the amino acid composition of the naturally occurring analogues. In the new pentadecapeptide all amino acids with aromatic side chains have been replaced. Formally the strictly observed pattern of alternating configurations of L- and D-amino acids has been broken for the first time. The question asked was this: Is it possible to replace all D-amino acids by GLYCINE without adverse effect on the conformation of the β-helix, the prerequisite for channel formation and ion transport across lipid bilayers? The answer is yes, glycine does adequately and functionally substitute for the D-amino acid residues of the naturally occurring analogs of the linear gramicidins. The data presently on hand are in support of the formation of a β-helical structure by HCO-(L-Ala-Gly)$_7$-L-Ala-NHCO$_2$CH$_2$OH as indicated by the detection of single channel characteristics for the molecule once embedded in the lipid bilayer. ii. HCO-(-L-Val-D-Val)$_7$-L-Val-NHCH$_2$CH$_2$OH and shorter chain segments of the molecule synthesized for studies on lipid bilayers and in experiments with neonatal and neoplastic tissue to probe for fetal resorption inducing and tumor growth inhibiting activity. Rationale: the naturally occurring linear gramicidins display both the activities indicated, however, not without side effects. The latter are attributed to the presence of amino acids with aromatic side chains. Such groups are no longer present in the newly designed molecule that has been synthesized. Other analogs of the linear gramicidins prepared and investigated: formyl-(L-tryptophyl-glycyl)-(L-tryptophyl-D-leucyl)$_6$-L-tryptophylethanolamide, formyl-(L-tryptophyl-D-leucyl)$_7$-tryptophyl ethanolamide, and fragments, e.g. (L-Phe-D-Leu)$_2$-L-Phe, for the synthesis of formyl-(L-phenylalanyl-D-leucyl)$_7$-L-phenylalanyl-ethanolamide. iii. Synthesis of Thyrotropin Releasing Hormone (TRH) - pyro-Glu-His-Pro-amide - for clinical studies. iv. Synthesis of Heterodetic Multicycle Peptides: 1. Nisin. Approaches to the synthesis of the biologically active (induced fetal resorption; inhibition of the growth of tumor cells) H$_2$N-terminal fragment of nisin call for the individual formation of Rings A, B, and C and the subsequent stepwise condensation of these structures. First requirements for the synthesis of the various rings were the successful access to the unusual structural feature seen in Ring A of nisin. The α,β-unsaturated amino acid DEHYDROALANINE is incorporated into suitable peptide structures via β-elimination of the appropriate precursors properly incorporated in the correct position in the form of an O- or S-substituted derivative of serine and/or cysteine, respectively. Great difficulty is associated with the preservation of the chirality of meso-lanthionine to be placed into Ring A. The most promising route established forms a sulfonium salt from S-alkyl-cysteine and β-halo-alanine, that is to be decomposed subsequently to give the thioether amino acid (in meso-form) without sacrifice in chirality. v. Synthesis of radio-labelled formyl- and tert.-butyloxycarbonyl-(L-phenyl-analyl-D-leucyl)$_2$-L-phenylalanine (vide supra, the segments for the synthesis of analogs of the linear gramicidins) for studies in chemotaxis. The formyl derivative is an agonist the tert.-butyl derivative an antagonist of chemotaxis (neutrophils). To establish the mechanism of action of the agonist and antagonist, p-halophenylalanine has been incorporated in

different positions of the peptides for catalytic dehalogenation to provide tritium-labelled derivatives. vi. Synthesis of enkephalin analogs for the exploration of agonist and antagonist properties and for physicochemical investigations.

Analysis: Peptides - Cinnamycin and Duramycin. Hydroxyaspartic Acid-Dehydroaspartic acid. The hydrolysates of cinnamycin and duramycin contained a contituent of unestablished identity that elutes between the erythro- and threoform of hydroxyaspartic acid. The unknown component was likely to be derived from hydroxyaspartic acid and possibly its dehydration product, namely dehydroaspartic acid. Comparison with an authentic sample of newly synthesized dehydroaspartic acid has now unequivocally established the identity of the unknown constituent in the hydrolysates of cinnamycin and duramycin. The relationship between hydroxyaspartic acid and dehydroaspartic acid (reversible formation via hydration and dehydration) may well be of physiological significance. Amino acids found in cinnamycin and duramycin and linked in other ways via β-elimination and addition reactions are lanthionine, β-methyllanthionine, lysinoalanine.

Analysis: Amino Acids - Osteogenesis imperfecta - Hydroxylysine. Special conditions have been developed for the separation of hydroxylysine and glycosides of this amino acids on the ion exchange column of the amino acid analyzer. The determination and quantitation of hydroxylysine and its glycosylated derivatives are to be developed to a tool of diagnosis for osteogenesis imperfecta.

Significance to Biomedical Research and the Program of the Institute:

The sequence of amino acids and the synthesis of peptides with unique structural features are prerequisites for the understanding of the effects exerted by certain of these moleculse on endocrine function. Amino acid assemblies capable of controlling the release of hormones are of foremose concern. The execution of well-controlled procedures of peptide synthesis provides molecular species for clinical applications aimed at fertility regulation.

Nisin, the H_2N-terminal fragment of nisin, and the linear gramicidins act on neonatal and neoplastic tissue by inducing fetal resorption in rodents and inhibiting the growth of tumor cells. The modes of action responsible for these effects are not known at present. Working hypotheses call for: (a) the addition of essential nucleophilic groups in the physiological environment across the double bond of unsaturated amino acids, such as dehydroalanine (cf. nisin and its fragments); (b) the disturbance of vital ion equilibria existing on either side of the biomembrane (cf. the ionophoric and transmembrane-channel forming properties of the gramicidins A, B, and C, and their synthetic analogs). α,β-Unsaturated amino acids may be vital to many physiological processes. The structural features seen in these unique amino acids are related to the amide and keto-function - both very well established structural elements of physiological consequence - and to the peptide bond - a linking feature of no lesser biological significance.

The amide function is seen at the carboxyl terminus of all structally established hormone releasing factors. It is also found in the same position of numerous other biologically active peptides. The group of neurophypophyseal peptides is a case in point. The use of dehydroalanine as vehicle for the attachment of peptides to carriers in solid phase peptide synthesis and the direct conversion of this amino acid to the amide function in the exo- and in endo-positions of the peptide chain are a development that benefited from the chemistry developed in this laboratory subsequent to the discovery of dehydroalanine and dehydrobutyrine in nisin and in subtilin.

The linear gramicidins are distinquished from all other know peptide structures by the regularly repeating pattern of L- and D-configurations. This gives rise to a unique hydrogen bond stabilized helix capable of embedding in lipid bilayers and biomembranes and the mediation of ion transport through the transmembrane channels formed. The effects of the native linear gramicidins and their synthetic analogs on neonatal and neoplastic tissue (vide supra) are believed to be the consequence of the physical properties indicated and brought about by the structural features intrinsic to these molecules.

The peptides for which chemotactic and anti-chemotactic properties have been demonstrated (vide supra) are partial sequences of analogs of the linear gramicidins. Assuming a mode of action different from that involving ion transport only, more subtle details of the relationship between structure and chemotaxis are to be investigated subsequent to the incorporation of radiolabel (dehalotritiation of phenylalanine).

The synthesis of partial sequences of hormones (and other physiologically important amino acid assemblies) that are responsible for antigenic activity provides important tools for the purpose of early diagnosis (radioimmunoassay) under normal and/or pathological circumstances.

Proposed Course of Project:

Improvements in the gram-scale synthesis of hormone releasing and hormone release inhibiting factors for fundamental and clinical investigations. Synthesis of H_2N-terminal protected peptide amides with properties antagonistic to the release of luteinizing and/or follicle stimulating hormone. Synthesis of analogs of the linear gramicidins with and without radiolabel for autoradiographic investigations and studies to elucidate the modes of action of these peptides in the various physiological effects they cause (action on neonatal and neoplastic tissue; embedding in membranes; exploration as chemotactic and/or anti-chemotactic agents). Synthesis of partial sequences and individual ring systems of nisin; exchange of amino acids in and incorporation of radiolabel into these peptides.

Synthesis of anlaogs of: (a) opioid peptides (enkephalin and endorphin) with the prime objective of providing antagonists; (b) chemotactically and anti-chemotactically acitve peptides with alignments of the amino acids that are patterned on partial sequences of the linear gramicidins. Incorporation of radiolabel (tritium via dehalotritiation) for the purpose of exploring proposed mechanisms of chemotaxis.

Development and improvement of: (a) methods for the synthesis of peptides; (b) the protection of the functional groups of amino acids (the large scale synthesis of the luteinizing hormone releasing factor is one of cases that made evident the inadequacies of existing protecting groups; in this particular situation none of the protections for the guanido group of arginine proved satisfactory; ornithine, suitably protected at the δ-amino group was employed as precursor; a guanidination reaction was executed subsequent to deprotection; several of the protecting groups under consideration are based on the chemistry of thioether and α,β-unsaturated amino acids); (c) Non-proteolytic techniques that are either applicable in general to the fragmentation of peptides and proteins or are conceived for the specific needs of establishing rare structural features, such as those seen in cinnamycin and duramycin. Correlation of identical structural components (e.g. α,β-unsaturated amino acids, lanthionines, lysinoalanine, and hydroxyaspartic acid) in peptides (nisin, subtilin, cinnamycin, duramycin) of strikingly different origin by the pursuit of phylogenetic questions.

Isolation, purification, and characterization of peptides of natural origin and chemical synthesis of peptides for the study of their inhibitory potential on intrauterine growth. Systematic screening of compounds of different chemical structure for activities on neonatal and neoplastic tissue. Spectroscopic investigation (nuclear magnetic resonance with different nuclei and circular dichroism) of the linear gramicidins and fragments of nisin in their interaction with lipid bilayers of known composition and adequately analyzed biomembranes. Conductance measurements and detailed investigations of the ion transport brought about by the gramicidins A, B, and C and synthetic analogs with amino acid exchanges. Isolation and characterization of subcellular constituents from developing tissue. Improvements in the design and construction of amino acid analyzers and peptide synthesizers.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00040-04 RR

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Experimental and Theoretical Studies of Hormone Receptor Interaction and Response Coupling

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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School of Medicine, Stanford University

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Endocrinology and Reproduction Research Branch

SECTION
Biophysical Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5	4.5	0.25

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A correlated series of experimental and theoretical studies regarding hormone receptor interactions has been undertaken. A number of model systems have been utilized, including the receptors for enkephalin, opiates, benzodiazepines, LHRH, and Insulin. By development of appropriate mathematical models, computer programs for curve fitting and data analysis, and of improved experimental designs, we have been able to characterize several of these ligand-receptor systems. Special attention has been given to the cases of 1) multiple ligands reacting simultaneously with multiple classes of sites, 2) the presence of multiple sub-sites on receptor areas; 3) the interaction of ligand-binding with an ionophore; 4) the interaction of receptors for different ligands on the same cell membrane.

PHS-6040
(Rev. 10-76)

Objectives:

This project is intended to develop both experimental and theoretical methods for analysis of hormone, neurotransmitter, and drug binding to their receptors. Methods are under study for characterization of the ligand-binding system (in terms of binding capacities, affinities, cooperativity factors, and any complex interactions), and the coupling of binding in response of the target cell (ionophore activation, second messengers and related phenomena).

Methods Employed:

Laboratory technique for binding of peptide hormones, steroid hormones, drugs, and neurotransmitters to their naturally accurring receptors, using appropriately radioactively labeled ligands. Also techniques of mathematical modeling, computer simulation, curve fitting, and Monte Carlo methods.

Major Findings:

The benzodiazepine receptor has been characterized. A number of experimental factors modify the behavior of this receptor. In particular chloride, iodide, bromide, and a series of other anions modulate the affinity of this receptor. Likewise GABA increases the affinity of this receptor, primarily due to a decrease in the dissociation rate. The effects of iodide and GABA are additive. Biccuculine, a GABA antogonist, can reverse the affect of both GABA and to some extent, iodide. Certain ionophore blocking agents (DIDS, DNDS) also modulate the behavior of the benzodiazepine receptor, as do selected purine and purine-analogs. This appears to be an ideal model system for study of interaction of multiple receptors on the cell surface, especially since it appears that both benzodiazepine and GABA are interacting with the same chloride anion channel. Studies are also continuing, in an attempt to isolate the naturally occurring endogenous ligand for the BZ receptor.

The opiate receptor: We are utilizing mathematical modeling studies, and new experimental procedures to attempt to characterize the heterogeneity of the opiate receptor in brain, vas deferens, and other tissues. This has required new experimental designs, involving use of multiple labeled ligand, and constructing displacement curves for a variety of unlabeled ligands which show specificity for various sub-types of the receptor. To assist the studies, new labeled analogs of both enkephaline and opiate have been designed.

A newly developed program, designated "SCAFIT", enables us to characterize ligand-binding systems, where data are available using multiple labeled ligands reacting with multiple classes of sites, with displacement curves for a variety of unlabeled analogs. This has been used to characterize the LHRH receptor on bovine and rat pituitary membranes.

These studies have shown that two newly developed analogs, D-Ser^{6}(t-Bu) des-gly^{10} NETH and D-Ala^{6}-des-gly^{10} NETH compete for the same sites as native LHRH. Further, the receptors show severe heterogeneity, consisting of two classes of sites of widely differing affinities. The affinity consistent for each of the three ligand reacting with both classes of sites have been estimated by least-squares procedures. The model has been used to make several predictions which are now being evaluated experimentally. Three studies indicate that the iodinated analogs serve as appropriate probes of the LHRH receptor.

The new method has been developed to characterize binding isotherms in terms of the limiting slopes and intercepts of the Scatchard plots or of the corresponding dose response curves (in terms of B/T vs T). Another new method has been developed to characterize binding systems in terms of a continuous distribution of affinities. This method, requiring a relatively simple and short computer program, should be widely applicable to characterization of a wide variety of systems. A third new method for characterizing ligand-binding systems has been developed: This describes the binding systems in terms of a "discrete affinity distribution". This may be implemented on computer programs "DISAFF", "SCAFIT", or a third program, "SCATFIT". In this approach, one sets the K values equal to pre-selected values, based on previous experimental data. This reduces the number of parameters which must be estimated, and thus vastly facilitates the curve-fitting procedures. These last three mentioned approaches should be extremely useful for characterizing steroidhormone receptor systems, particularly in the context of screening for estrogen, progesterone, androgen and glucocorticoid receptors in Breast Carcinome and other hormonally responsive tumors.

Two computer programs have been developed for characterization of ligand binding systems which can be described by the "De Myets" model. This permits statistical estimation of the affinity, binding compacity, cooperativity factor, and nonspecific binding. Methods have been developed for combining results from multiple curves within an experiment, from multiple patients, and from multiple experiments in order to obtain the most reliable estimates of parameters.

Significance to Biomedical Research and the Program of the Institute:

Much current research interest centers around the binding of hormones, drugs and neurotransmitters to their naturally occurring receptors. The models which have been developed here should be generally applicable to endocrinology and to neurobiology. Further, these models are now sufficiently well advanced, so that they can be used to make predictions in specific systems, and to assist significantly in the design of experiments to discriminate among the possible mechanisms of action.

Proposed Course:

We expect that the models currently under study will continue to evolve in collaboration with several experimentalists at the National Institutes of Health and throughout the country. The models will be progressively refined and used as guides to experimentation. As new experimental results become available, the models will be developed further. In this process, a series of computer programs will continue to be developed for routine use in analysis of binding systems. We expect to have considerable increased application of these models to systems such as protein and polypeptide hormones, e.g., angiotensin, catecholamines, and steroid receptors.

By design of new ligands (analogs of enkephaline, LHRH, diazepam, etc.) we expect to be able to generate new experimental results which can be used to test hypothesis generated on the bases of existing data and models. We believe that some of these new probes will produce new structure-activity information unobtainable by any other method.

Publications:

DeLean, A. and Rodbard, D.: Kinetics of Cooperative Binding. In O'Brien, R.D. (Ed.): The Receptors. Volume 1, Plenum Press, New York, 1978, pp. 142-192. Murakami, T., Brandon, D., Rodbard, D., Loriaux,

Murakami, T., Brandon, D., Rodbard, D., Loriaux, D.L., and Lipsett, M.B.: Glucocorticoid Receptor in Circulating Mononuclear Leukocytes. Endocrinology. 104(2):500-505, 1979.

DeLean, A., Munson, P. J., and Rodbard, D.: Simultaneous Analysis of Families of Sigmoidal Curves: Application to Bioassay, Radioligand Assay, and Physiological Dose-Response Curves. American Journal of Physiology: Endocrinology, Metabolism, and Gastrointestinal Physiology. 4:E97-E102, 1978.

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Thakur, A.L., and Rodbard, D.: Graphical Aids to Interpolation of Scatchard Plots and Dose-Response Curves. Journal of Theoretical Biology, in press.

Costa, T., Rodbard, D., Pert, C.B.: Interaction between the benzodiazepine receptor, the GABA receptor, and a common anion channel. In DeLisi, C. and Blumenthal, R. (Eds.): Physical Chemical Aspects of Cell Surface Events in Cellular Regulation. Developments in Cell Biology, Volume 4, Elsevier/North Holland, Amsterdam, New York, pp. 37-52, 1979.

Wachslicht-Rodbard, H., Gross, H., Rodbard, D., Ebert, M., and Roth, J.: Increased insulin binding to erythrocytes in anorexia nervosa: Restoration to normal with refeeding. New England Journal of Medicine 300:882-887, 1979.

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DeLean, A.: Analysis of Receptor Response Coupling by Means of a Logit-Logit Transformation. Journal of Biological Chemistry, in press.

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Murakami, T., Brandon, D., Rodbard, D., Loriaux, D.L., and Lipsett, M.B.: Glucocorticoid Receptor in Polymorphonuclear Leukocytes: A simple method for leukocyte glucocorticoid receptor characterization. Journal of Steroid Biochemistry, May 1979, in press.

Munson, P.J. and Rodbard, D.: Computer Modeling of Several Ligands Binding to Multiple Receptor, Endocrinology, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00041-04 RR

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Cardiovascular Effects of Thyroid, Hormones, Catecholamines and Cardiotoxic Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	D. Rodbard	Chief, Section on Biophysical Endocrinology	ERRB, NICHD
Other	B. Bercu	Clinical Associate	NPMB, NICHD

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LAB/BRANCH
Endocrinology and Reproduction Research Branch

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☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effects of thyroid hormones and beta adrenergic agonists on target tissues may be evaluated by noninvasive monitoring of cardiovascular function. New techniques have been developed and evaluated for this purpose. The QKD interval, i.e., the interval between the onset of ventricular depolarization and the arrival of the pulse wave at the brachial artery provides a rapid, innocuous, precise, reproducible, noninvasive technique for evaluation of thyroid cardiovascular and/or catecholamine status. This has been used for diagnosis and monitoring of therapy for patients with thyrotoxicosis, hypothyroidism, pheochromocytoma, and in the titration of effects of beta adrenergic blockers such as propranolol. This method has now been applied to evaluate thyroid function in children with cystinosis, and appears to indicate that patients are generally euthyroid.

PHS-6040
(Rev. 10-76)

Objectives:

Develop and evaluate methods for noninvasive monitoring of thyroid status, catecholamine stimulation or blockade, and myocardial contractility in patients with drug-induced or other cardiomyopathies.

Methods Employed:

A new method has been developed called "Sphygmo-Recording." The electrocardiogram, phonoarteriogram, and the pressure from the blood pressure cuff are fed into a specially designed electronic apparatus which automatically displays the calibrated contour of the arterial pressure wave on the phase of a storage oscilloscope. This is then photographed by Polaroid camera. This electronic instrumentation has also been utilized for improved recording of heart sounds, carotid sounds, thyroid bruits and a variety of other cardiovascular signals.

Major Findings:

There has been some question regarding the thyroid status of patients with cystinosis. Accordingly, the QKD measurements have been obtained in a series of approximately 10 patients with this disease. In order to evaluate these, normal ranges needed to be established in children of the same age and/or of the same height, weight, and hence, physical dimension between the heart and the location of the transducer. This has led to the collection of data to evaluate the normal QKD interval in children and to study the relationship between height and QKD interval.

Significance:

Despite the proclivity of chemical tests (particularly radioimmunoassays) for thyroid hormones, there is no generally available, reliable measure of thyroid effects on the target tissues for routine clinical use. The present study provides one such measure of thyroid effects on the end organ. In addition, it is useful in monitoring of therapy for pheochromocytoma, and for beta adrenergic stimuli and blockers. This method is useful in evaluating the cardiotoxicity of Adriamycin and related cancer chemotherapeutic agents.

Proposed Course:

The use of this technique for monitoring thyroid status is now well established, but it retains research interest in special cases. Accordingly, this measurement is to be used selectively in special cases, where a reliable measure of end-organ function is necessary, in addition to measurement of plasma levels of thyroxine and tri-iodothyranine.

Publications:

Bercu, B. B., Haupt, R., Johnson Baugh, R., and Rodbard, D.: The Pulse wave arrival time (QKD interval) in normal children. J. Pediatrics. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00042-04 RR

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Mathematical and Statistical Theory of Gel Electrophoresis and Gel Filtration

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	David Rodbard	Chief, Section on Biophysical Endocrinology	ERRB, NICHD
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	Betty Chen	Staff Fellow	ERRB, NICHD
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Massachusetts Institute of Technology, Cambridge, Massachusetts

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SECTION
Biophysical Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Mathematical and statistical principles of polyacrylamide gel electrophoresis, SDS-polyacrylamide gel electrophoresis, isoelectric focusing, gel filtration, density gradient sedimentation, and related physical-chemical techniques have been developed. This is to permit optimization of conditions for fractionation of protein and polypeptide hormones, proteoglycans, hormone receptors and other macromolecules. Methods and computer programs have been developed to permit improved statistical estimation of molecular weights derived from these techniques. Further, general computer programs have been developed for analysis of the concentration profiles which arise in electrophoresis, chromatography, sedimentation and other physical-chemical techniques. These programs should be extremely useful for measurement of diffusion coefficients, bandwidth, and resolution.

PHS-6040
(Rev. 10-76)

Objectives:

Improvement of the performance of physical-chemical techniques used for the analysis and purification of protein and polypeptide hormones.

Methods Employed:

Polyacrylamide gel electrophoresis, gel filtration, sucrose density gradient sediment, sucrose density electrophoresis of cells and proteins, isoelectric focusing. In particular, polyacrylamide gel electrophoresis and isoelectric focusing is performed with continuous scanning of the concentration profile with computation of relevant molecular parameters.

Major Findings:

Beta lactoglobulin B has been studied in detail using continuous scanning polyacrylamide gel electrophoresis. This has permitted an evaluation of the factors which control band width, and hence resolution under routine conditions of gel electrophoresis. In particular, the effect of gel concentration, protein load, ionic strength, current density, and duration of electrophoresis have been studied. We find that the concentration profiles are compatible with a single Gaussian peak. The square of the standard deviation of peak width is linearly related with time over the major period of interest. Thereafter the relationship becomes flatter, as would be expected, as the protein concentration within the zone decreases, and hence, the effective diffusion coefficient decreases. The apparent diffusion coefficient is linearly related to protein load and to current density. Accordingly this can be extrapolated back to 0 load and 0 current density to obtain an idealized limiting value. Likewise, there is a linear relationship between the logarithm of the apparent diffusion coefficient and gel concentration. The slope of this relationship is identical with the slope of the relationship between a logarithm of mobility and gel concentration. Accordingly, the diffusion coefficient is directly proportional to mobility, as was predicted for the ideal case. This indicates that the protein under study is not subject to microheterogeneity, in which case the apparent diffusion coefficient would be related to the square of the protein mobility.

We have also studied the factors affecting the band-width and hence resolution in both continuous buffer systems and multiphasic buffer systems. We have been able to establish under what conditions the use of multiphasic buffer systems result in sharper initial band width and hence improved resolution.

A new computer program has been developed for the automatic analysis of concentration profiles which arise in electrophoresis, chromatography, and sedimentation. Usually such profiles consist of one or several Gaussian peaks, superimposed on a base line. Algorithms have been developed for automatic, computerized peak detection. This is followed by characterization of the peaks in terms of the mean location (centroid or center of gravity) area

and standard deviation. A nonlinear least-squares curve fitting program is utilized to describe the curves as the summation of several overlapping Gaussian components. This program, written in the BASIC language and with portions in the SAIL language, are presently available on the DEC-system 10 computer. However, these programs are readily adaptable to the larger desk-top computers, and hence can be distributed widely to workers in endocrinology and other fields which employ protein fractionation and analysis. In particular, this will result in improved methods for measurement of the area under the peak, which in turn is utilized for construction of Scatchard plots in a variety of applications. This should improve our ability to detect factors which result in a minor shift in the position of the peak, and in better characterization of profiles wherein the peak of interest occurs as the "shoulder" on adjacent peak.

A new method has been developed for estimation of molecular weights on SDS-polyacrylamide gel electrophoresis when using pore-gradient gels. We have developed programs to compute the relationship between log(MW) and log(%T), where %T represents the gel concentration at the final position on the gel which the protein reaches at the end of the electrophoretic run. However, a theoretical study shows that this linear relationship is only an approximation. An exact physical-chemical model has been developed for analysis of this system, and has been implemented using non-linear least-squares curve fitting procedures. These methods have been applied to the analysis of basic myelin proteins. In general, the precision of this method is $\pm$ 10%, which compares favorably with most other methods for estimation of molecular weights.

A new method has been developed for estimation of the calibration curve for sucrose density gradient sedimentation. By applying program "CEPHAS" to the analysis of density gradient sedimentation, one can improve the precision of estimation of the position of the protein peaks, by approximately a factor of two. This improvement applies both to the position for the peak within a given tube, the variability between tubes within a given run of the ultracentrifuge, and the variability between runs. By virtue of computerization, variability is reduced by approximately a factor of two. This corresponds to approximately a two-fold improvement in the precision of the estimate of the sedimentation coefficient. Appropriate least-squares computer programs have been developed for estimation of sedimentation coefficient ("s") using the popular method of Martin and Ames. It has been demonstrated that the popular use of 2, 3, or 4, point to define the standard curve results in enormous 95% confidence limits for the "s" value for unknown proteins. When utilizing 2, 3, or 4 standard proteins, one can estimate the "relative sedimentation coefficient", i.e. the position of an unknown relative to a few arbitrarily selected standards. However one can not estimate the "s" value with precision, because there is the possibility that the standards may be nonrepresentative of the underlying population of all globular proteins.

Significance to Biomedical Research and the Program of the Institute:

The use of polyacrylamide gel electrophoresis, gel filtration, density gradient sedimentation, has been extremely popular in the analysis and characterization of protein and polypeptide hormones, the receptors for these hormones, and likewise the receptors for steroid hormones. Indeed these tools are indispensable. Accordingly, improved techniques for the calculation of the results, and the identification of the various factors describing the peak (e.g., skewness, kurtosis or shape) will result in improved extraction of information from prior experiments and improved design of future experiments. Further, these computer programs can save hours of technician time and therefore contribute to the efficiency of operation of the programs of the Institute. The identification of factors which affect band-width and hence resolution in polyacrylamide gel electrophoresis and isoelectric focusing is important in optimizing conditions for preparative PAGE. Further, this makes it possible to measure or at least to estimate the free diffusion coefficient for protein. In turn this permits estimation of molecular radius and derived parameters such as the fractional coefficient and axial ratio.

Proposed Course:

The project, involving both theoretical and experimental aspects, will continue in an attempt to develop more useful methods for characterization of protein and polypeptide hormones, and receptors for protein and steroid hormones. The use of continuous scanning of electrophoresis columns to permit estimation of the diffusion coefficient, molecular radius and microheterogeneity of size and/or charge is to continue, together with the corresponding theoretical studies necessary for the automated computerized analysis of results. The results of these studies will be applied to practical problems in the fractionation and preparation of peptide hormones and receptors for peptide hormones. A mathematical theory of isoelectric focusing is under development.

Publications:

Chen, B., Rodbard, D., and Chrambach, A.: Polyacrylamide Gel Electrophoresis with Continuous Optacle Scanning using Multiphasic Buffer Systems: The Stack. Analytical Biochemistry 89: 596-608, 1978.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00043-04 RR

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Mathematics and Statistics of Radioligand Assays

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Other	M. Jaffe	Bio Aid	ERRB, NICHD
	B. Cole	Programmer	DMB, DCRT

COOPERATING UNITS (if any)

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Biophysical Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.25	1.25	1

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The mathematical and statistical theories of radioimmunoassay have been under progressive development, refinement, and testing. In the past year, attention has focused on immunoradiometric assays, two-site immunoradiometric (labeled antibody) assays (sandwich assays), enzyme-mediated immunoassay techniques (EMIT), enzyme-linked immunosorbent assays (ELISA), and the labeled antibody membrane assay (LAMA). The chemical and mathematical equations describing these systems have been developed and used for computer simulation. Results provide a theoretical basis for the observed "paradoxical high dose hook effect" which occurs in many of these systems and suggests ways for improvement of these assay systems. The mathematical theory of optimization of RIA has been tested in practice and existing computer programs have been shown to be valid and useful guides to the experimentalist. Several new statistical methods for analysis of radioimmunoassay results have been developed. New computer programs have been developed for quality control of RIA, both within laboratories and for regional and world-wide collaborative studies.

PHS-6040
(Rev. 10-76)

Objectives:

Development of mathematical and statistical methods for evaluation of results in radioimmunoassay (RIA) systems for optimization of radioligand assays, and for statistical quality control of RIA laboratories.

Methods Employed:

Mathematical modeling, statistical analysis, computer simulation and practical laboratory radioimmunoassays for a variety of hormones, including hCG, estradiol and human growth hormone.

Major Findings:

EMIT: The reaction scheme for EMIT has been formulated, the corresponding differential equations have been written, and these have been solved utilizing numerical techniques, employing the MLAB program of the DEC-System 10 computer at the National Institutes of Health, DCRT. This permits analysis of the individual factors which affect the assay, including the antibody concentration, labeled (enzyme tagged) antigen, incubation times, and the interaction of these factors. Further, we have studied the effect of order of addition of the reagents, e.g., delayed addition of labeled antigen, delayed addition of substrate, etc. Also the roles of the 4 incubation times which describe the system have been studied in detail, both for systems in which antibody binding to ligand labeled enzyme results in a decrease in the Michaelis-Menten constant (Km), or in systems where the antibody-ligand-enzyme complex results in a decrease in the Vmax of an enzyme system. Computer programs have been developed to facilitate optimization either in terms of sensitivity, or in terms of precision at any desired target dose level.

Methods for calculation of the sensitivity (minimal detectable dose) of radioligand assays and related techniques have been developed and refined. Programs have been developed for the routine analysis of radioligand assay systems, involving simultaneous analysis of all of the dose response curves in the system. This permits refined testing for identity of standard and unknowns, not only in regard to slope, but also in regard to minimal and maximal responses, corresponding to the lower and upper asymptopes of the dose response curve. New programs have been developed for fitting the mass-action law, in the case of heterogeneity of receptor, or when multiple ligands combine simultaneously with multiple receptor sites. The statistical analyses of these and similar programs have been improved to permit refined testing of goodness of fit. Computer programs have also been adapted from the IBM 370 to the DEC-system 10, and in turn these programs have been simplified so that they can operate, in some cases on a hand-held calculator such asmP u[Texas Instrument 59. In this manner, programs which once required relatively sophisticated computing facilities are now available to laboratories in developing countries throughout the world where such analyses were heretofore impossible. This adaptation of software has been in collaboration with the program for quality control in reproduction research sponsored by the World Health Organization.

New methods have been developed for testing of parallelism of standard and unknown dose response curves, using a variety of rigorous statistical techniques. Several of these techniques are also readily adaptable to small desk-top or even hand-held calculators.

New programs have been developed for dose interpolation for radioimmunoassays. These involve use of splines, orthogonal polynomials, sums of hyperbolas, etc. We have developed methods for fitting of splines which are constrained to be parallel everywhere and monotonic. These programs are useful not only for radioimmunoassay, but for a variety of other data analysis applications as well. We have made further refinements in our programs for optimization of conditions for radioimmunoassay, two-site immunoradiometric assay, and enzyme labeled assays.

The characterization of the affinity of an antiserum is usually undertaken in the context of 1, 2, or 3 discreet classes of sites. We are now developing mathematical methods for characterization of these affinity profiles, in the context of a continuous distribution of affinities. This has required development of new computer programs, which should be generally useful to immunologists as well as to routine practitioners of radioimmunoassay.

New methods have been developed for comparison of results between two assays methods. Use of simple linear regression for this purpose is inappropriate and can be seriously misleading. One must take into account the errors in method A, the errors in method B, nonuniformity of these errors, and any interactions or correlations between these errors. The necessary computer programs have been developed for this type of analysis. This can be used for comparison of assay performance after a change of a single reagent (e.g., an antiserum), for comparison of two different methods within a given laboratory, for comparison of the same method or kit in two different laboratories, or comparison of different methods in different laboratories. This is an important tool for quality control. Several other new programs have been developed for RIA quality control. These provide the frequency distribution analysis of the slopes of samples within an assay, the frequency distribution of the counts recorded and frequency distribution of potency estimates for unknown samples within an assay.

Significance to Biomedical Research and the Program of the Institute:

This laboratory continues to be the major supplier of computer programs for analysis of radioimmunoassay results to the entire country, and to most of the world. In turn, dissemination of these programs results in "feedback" which enables us to revise and extend these programs so that they remain current and "state of the art." Further, numerous interesting applications become available, not only in the context of radioimmunoassay, but also in the context of radio receptor assay and receptor systems in general. Further, these programs provide automation, considerable saving of professional and

technician time to members of the Reproduction Research Branch and National Institute of Child Health and Human Development, and provide estimates of the precision and reliability of the results of radioimmunoassays, radio receptor assays and bioassays. As a result, experimental designs can be optimized with considerable saving in cost of reagents and professional time. Further, by making apparent the results of subtle improvements in the assay, these programs facilitate refinement of assays to stages which would otherwise not be possible.

Publications:

Rodbard, D.: Statistical Estimation of Minimal Detectable Dose (Sensitivity) for Radioligand Assays. Analytical Biochemistry. 90(1):1-12, 1978.

Rodbard, D., and Tacey, R. L.: Radioimmunoassay Dose Interpolation Based on the First-Order Mass-Action Law with Antibody Heterogeneity: Exact Correction for Variable Mass of Labeled Ligand. Analytical Biochemistry. 90(1):13-21, 1978.

Yanagishita, M., and Rodbard, D.: Computer Optimization of Radioimmunoassays for hCG and Estradiol: An Experimental Evaluation. Analytical Biochemistry. 88:1-19, 1978.

Rodbard, D.: Mathematics and Statistics of Radioimmunoassay and Radioligand Assays. In Hamilton, R.G. (Ed.): Syllabus, The Clinical Radioassay Society Convention '79, Washington, D.C., 1979, pp. 43-85.

Munson, P. J., and Rodbard, D.: An Elementary Component of Variance Analysis for Multi-Center Quality Control. In Proceedings Symposium on Radioimmunoassay and Related Procedures in Medicine, 1977. International Atomic Energy Agency, Vienna, 1978, Volume 2, pp. 105-125.

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Rodbard, D., Munson, P.J., and DeLean, A.: Improved Curve-Fitting, Parallelism Testing, Characterisation of Sensitivity and Specificity, Validation, and Optimization for Radioligand Assays. In: Radioimmunoassay and Related Procedures in Medicine, International Atomic Energy Agency, Vienna, 1978, Volume 1, pp. 469-504.

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Munson, P.J., Rodbard, D.: Statistical Methods for comparison of two assay methods (weighted linear regression with errors in both variables). In Wilson, D.W. (Ed.): Quality Control of Radioimmunoassays, Tenovus Research Institute Symposium, 1979, in press.

Rodbard, D., Cole, B., and Munson, P.J.: The Need for Innovative Approaches to Radioimmunoassay Quality Control. In Wilson, D.W. (Ed.): Quality Control of Radioimmunoassays, Tenovus Research Institute Symposium, 1979, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00146-04 RR

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Structure and Function of Chorionic Gonadotropins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	H. C. Chen	Senior Investigator	ERRB, NICHD
Other	S. Matsuura	Visiting Associate	ERRB, NICHD
	M. Ohashi	Visiting Fellow	ERRB, NICHD
	G. D. Hodgen	Chief	PRB, NICHD

COOPERATING UNITS (if any)

Contraceptive Development Branch, CPR, NICHD

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.2	3.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The topics of the present research programs are: (1) the isolation and characterization of human chorionic gonadotropin-like (hCG-like) substance in tissues and biological fluids of normal subjects; (2) the generation and characterization of specific antisera against synthetic peptides based on the unique chemical structure of the hCGβ subunit; (3) the development of picogram quantity assay method specific for hCG devoid of hLH interference; (4) the purification of chimpanzee chorionic gonadotropin (chCG) for the biological, chemical and immunological characterization.

PHS-6040
(Rev. 10-76)

Objectives:

To establish the presence of hCG-like substance in tissues and fluid of normal subjects and to demonstrate its biological role in reproduction and development.

To search for antigens that generate hCG-specific antisera permitting the development of improved immunoassay methods in monitoring a low level of hCG activity and the preparation of immunoabsorbents for concentration and purification of hCG and hCG-like substances.

To isolate, purify and characterize chorionic gonadotropin from non-human primates in search of chemical homologues in order to determine the basis and limit of employing primates as a model for the study of human reproduction.

Methods Employed:

Solid phase peptide synthesis, amino acid analysis, chromatographic techniques (i.e., gel, ion exchange, partition and bioaffinity), rat uterine weight assay, immunization of rabbits, radioimmunoassay, radio-ligand receptor assay, interstitial cell testosterone production assay, high speed digital computer analysis, spectrophotometry and spectrofluorometry.

Major Findings:

1. Critical examination showing the presence of hCG-like substance in pituitary.

In the previous report, it has been shown that a partially purified glycoprotein fraction of human pituitary as well as the urinary extracts of postmenopausal women contain an hCG-like substance based on criteria, namely (1) immunoreactivities in specific radioimmunoassay system for hCG including one specific to the unique carboxyl-terminal sequence of the hCGβ subunit, (2) competition with hCG in a gonadal receptor assay, and (3) co-elution with ^{125}I-hCG in Sephadex G-100 gel chromatography.

A purified glycoprotein fraction from human pituitary, whose Sephadex G-100 gel chromatographic behavior resembled hCG was further purified by an immunoaffinity chromatography using a purified γ-globulin fraction that contained only those antibodies reactive to the terminal dodecapeptide of the hCGβ subunit. Analysis of the immunoabsorbed fraction by isoelectrofocusing on polyacrylamide gel (pH range of ampholytes, 3-6) revealed a pattern closely resembling that of a highly purified hCG (Lot CR119) with pI' of 4.4 to 5.1 as judged by an anti-hCG radioimmunoassay and the *in vitro* mouse interstitial cell testosterone production assay. The pooled fraction from the isoelectrofocusing experiment showed a peak coincided with ^{125}I-hCG (CR119) in Sephacryl S200 gel chromatography. Thus, the hCG-like substance identified in human pituitary is shown to possess all distinct properties of hCG.

2. Production and characterization of hCG-specific antisera.

New antisera against (i) N^{α}-acetyl-N^{ε}-dinitrophenyl-tricontapeptide (hCGβ residues 116-145), (ii) N^{α}-Gly_5-pentatricontapeptide (residues 111-145), and (iii) $desLys^{122}$,Gly^{122}-pentatricontapeptide (residues 111-145) have been produced. None of these antisera binds ^{125}I-hCG in a RIA system. Comparative studies of antibody recognition sites in three antisera: anti-hCGβ-tricosaglycopeptide (H93), anti-hCGβ-N^{α}-acetyl-tricontapeptide (H114) and anti-hCGβ-N^{α}-acetyl-N^{ε}-dinitrophenyltricontapeptide (H134), have revealed a remarkable qualitative similarity in antigenic sites which reside at the terminal pentadecapeptide region. Two binding loci, namely Pro-Gln, the terminal dipeptide and the sequence Arg-Leu-Pro-Gly (residues 133-136) dominate the antigenicity. The remaining amino-terminal portion of the sequence may be either non-antigenic in itself or in a folded conformation unable to elicit immunogenicity. The qualitative difference as compared to the H93 antiserum and H114 antiserum indicated that the latter exhibited twice the affinity toward the synthetic peptides suggesting that the masking of the second locus by carbohydrate moeities in the antigen resulted in decreased antigenicity. On the other hand, the restriction on the first locus as in the case of the H134 antiserum, where carboxyl-groups of the peptide are only allowable point of attachment to the carrier protein, also resulted in decreased antigenicity. Collectively, cooperative action of two loci is required to achieve the maximal binding affinity for this type of hCG-specific antisera.

3. Development of immunobioassay.

A solid phase immunoabsorbent has been prepared by coupling a purified γ-globulin fraction specific to the carboxyl-terminal dodecapeptide of the hCGβ subunit to Sepharose gel. This immunoabsorbent was used to concentrate hCG and hCG-like substance in tissue extracts and biological fluids and also to exclude hLH. HCG and hCG-like substance was extracted from the gel by a volatile buffer, concentrated and subjected to a highly sensitive in vitro mouse interstitial cell testosterone production bioassay. This procedure retains high specificity for hCG with increased sensitivity up to 50 folds for anti-hCGβ RIA and 2500 folds for anti-hCGβ-carboxyl-terminal peptide RIA.

4. Purification of chimpanzee chorionic gonadotropin.

ChCG was isolated from the kaolin-acetone extract of pregnancy chimpanzee urine. After batchwise absorption to concanavalin A-Sepharose and followed by the column chromatography on DEAE-Sephadex, the product (50 mg) exhibited in vivo potency of 6000 IU/mg (2ISTD, hCG) when assayed by either the rat uterine weight or the ovarian ascorbate bioassay methods. One less acidic fraction presumably low in sialic acid content possessed little in vivo biological activity but high in both in vitro biological and immunological activities. A large scale purification of these two fractions is in progress.

Significance to Biomedical Research:

1. The evidence that hCG-like substance is presented in the pituitary of

normal human subjects is enhanced by sequential examinations of incorporating biological, physico-chemical and immunological methods, which remove the potential ambiguity that can arise from the protease action.

2. The identification of antigenic sites specific to hCG enables us to design effective and practical antigens for the development of immunological assay systems. Large-scale production of these antisera can be employed to prepare an immunoabsorbent for the development of immunobioassay. It is the first time that a truly specific method allows an accurate measurement in picogram quantity of hCG. Usefulness of this method in experimental and clinical investigation is apparent.

3. Availability of purified chCG permits investigation on biological, chemical and immunological characteristics in comparison with hCG. The understanding based on these studies is essential for accessing the value and limitation in establishing a non-human primate model in human reproduction research.

Proposed Course:

1. To continue isolation and purification of the hCG-like substance from urine and pituitary of normal subjects by incorporating conventional chromatographic methods with a highly effective and specific immunoaffinity method and a high performance liquid chromatographic technique.

2. To design and synthesize polypeptides analogous to the carboxyl-terminal portion of the hCGβ subunit which allow an extended conformation in an attempt to increase antigenicity and specific binding affinity.

3. To produce, purify more hCG-specific antisera against the synthetic peptides. These antisera will be distributed to biomedical scientists through the National Pituitary Agency.

4. To collect, determine the content of hCG or hCG-like substance in tissues and biological fluids under physiological and pathophysiological conditions.

5. To study biological, chemical and immunological homologues between hCG and chCG and to purify 50-100 mg of chCG to be used as the WHO radioimmunoassay/bioassay standard.

Publications:

Matsuura, S., Ohashi, M., Chen, H. C., and Hodgen, G. D.: A Human Chorionic Gonadotropin-Specific Antiserum Against Synthetic Peptide Analogs to the Carboxyl-Terminal Peptide of Its β-Subunit. Endocrinology 104: 396-401, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00147-04 RR

PERIOD COVERED
October 1, 1978 - September 30, 1979.

TITLE OF PROJECT (80 characters or less)

Mechanism of Action of Gonadotropins and of ACTH.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	Maria L. Dufau	Chief, Section on Molecular Endocrinology	ERRB, NICHD
	Kevin J. Catt	Chief	ERRB, NICHD
Other	Keiko Hayashi	Visiting Fellow	ERRB, NICHD
	Graciela Sala	Visiting Fellow	ERRB, NICHD
	Ernesto Podesta	Visiting Associate	ERRB, NICHD
	Angela Solano	Visiting Fellow	ERRB, NICHD
	Selva Cigorraga	Visiting Fellow	ERRB, NICHD

COOPERATING UNITS (if any)

None

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Sections on Molecular Endocrinology and Hormonal Regulation

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	3.7	0.8

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The broad goal of this project is to understand the steps involved in the hormonal control of steroidogenesis. This includes the metabolic events that follow stimulation of trophic hormone receptors in the Leydig cells, luteal cell and adrenal fasciculata cells by gonadotropins and ACTH. Studies were focused on the interaction of hormones with their receptor, and on analysis of coupling mechanism between receptor occupancy and androgen, progesterone or corticosterone synthesis. The two major questions examined are (1) the relationship of receptor occupancy to cyclic AMP and protein kinase activation during acute dose-related increases in steroid production and trophic actions of gonadotropin; (2) the sequence of events which leads to stimulation of enzymes of the steroidogenic cells. This program has relevance to reproductive endocrinology and for the understanding of the mechanism of action of peptide hormones upon target cells.

PHS-6040
(Rev. 10-76)

Objectives:

To define the mechanisms of action of luteinizing hormone and ACTH upon steroidogenesis and growth in gonadal (Leydig and luteal cells) and adrenal fasciculata cells.

Methods Employed.

Purification of Leydig cell by Metrizamide density gradients. Characterization of LH receptor by binding analysis in cell membranes and intact Leydig and luteal cells. Measurements of extracellular, intracellular cAMP, and cyclic GMP and of endogenous cAMP bound to cAMP receptor protein (or regulatory subunit of protein kinase) by radioimmunoassay. Binding studies of [^{3}HcAMP] and [^{3}HGMP] to cAMP and cGMP receptor protein: assay of binding capacity, affinity, and kinetic studies. Protein kinase assay. Gel filtration, sucrose gradient centrifugation, ion exchange chromatography, DEAE cellulose and polyacrylamide gel electrophoresis for characterization of protein kinase holoenzyme and regulatory subunits. Thin-layer chromatography on alumina and silica gel for characterization of steroids. Binding studies of ^{125}I-cholera toxin to purified Leydig cells. Culture of Leydig cell tumors. Electron microscopy and light autoradiography. Polyacrylamide and S.D.S. gel electrophoresis.

Major Findings:

a) Regulation of Steroidogenesis by ACTH in Isolated Adrenal Cells.

The apparent dissociation between steroidogenesis and cyclic AMP during ACTH action has been recently resolved by a detailed analysis of cyclic AMP production and binding to receptors in ACTH-stimulated adrenal fasciculata cells. Analysis of the relationships between cyclic AMP, cyclic GMP and steroidogenesis during in vitro stimulation of adrenal cells by low concentrations of ACTH indicated that cyclic AMP but not cyclic GMP is involved in the acute steroidogenic response to ACTH, and that the effect of high concentrations of cyclic GMP derivatives on steroidogenesis occur through activation of cyclic AMP receptor with low efficacy.

Further studies were conducted to characterize the adrenal fasciculata cell protein kinase and cyclic AMP receptors by gel electrophoresis and DEAE cellulose chromatography, and to evaluate the occupancy of regulatory subunits after dissociation of the holoenzyme by endogenous cyclic AMP formed during ACTH action. We have demonstrated that the rat adrenal fasciculata cells contains two forms of cyclic AMP-dependent protein kinase with MWs of 160,000 (H_1) and 106,000 (H_2) respectively, and two regulatory subunits MWs 90,000 (R_1) and 10,000 (R_2). A serial decrease in the available cAMP receptor sites, corresponding to the protein kinase holoenzymes, was observed in the ACTH range that caused a dose-related increase of corticosterone production, and at supramaximal steroidogenic doses of ACTH. Simultaneously increased occupancy of both regulatory subunits binding sites was observed at low ACTH concentrations. ACTH action on steroidogenesis is mediated by cyclic AMP-dependent

activation of both protein kinase.

b) Hormonal Activation of Protein Kinase in Isolated Leydig Cells.

The characteristics and hormone-induced activation of protein kinase in testicular Leydig cells was analyzed as in the adrenal by polyacrylamide gel electrophoresis of holoenzymes and regulatory subunits in extracts of hCG stimulated Leydig cells. Activation induced by low gonadotropin concentration that produced a dose-related response in steroidogenesis were observed in the predominant cytosolic 6.2S holoenzyme, which was completely dissociated during stimulation of Leydig cells with steroidogenic doses of hCG (1 to 20 pM). In contrast, dissociation of the less abundant 4.0S holoenzyme was observed only with the highest hormone concentration. Thus, at difference with the adrenal where both holoenzymes were activated by low ACTH doses in the Leydig cell, there is a differential activation of cyclic AMP-dependent protein kinase during hormone action.

c) Regulation of Steroidogenic Enzymes.

The role of trophic hormones and steroid metabolites in the maintenance of steroid enzymes has been investigated. A partial defect in testicular enzymic activity was found responsible for the impaired testosterone responses of rat Leydig cells desensitized by intravenous hCG or sc LHRH that caused more than 50% loss of LH receptors. Accumulation of progesterone and 17OH-progesterone in blood and incubated Leydig cells of desensitized animals in vitro localized the major steroidogenic block at the conversion of 17OH-steroids to androgens. In vivo development of the steroid block was not coincident with the loss and return of LH receptors suggesting that the steroidogenic block was not only due to receptor depletion. A role of estradiol in enzyme regulation in vivo was suggested by acute elevations of testicular E_2 and by the ability of an estrogen antagonist (Tamoxifen) to prevent the reduction of testosterone responses caused by hCG or LHRH.

In contrast to the findings described above, desensitized cells with moderate receptor depletion (< 50% loss) show no steroidogenic lesion but an increase in the steroidogenic capacity of moderately receptor-depleted Leydig cells. The observed accumulation of 17-hydroxylated intermediates in such cells probably reflects the production of steroids in excess of the enzymatic capacity of 17a hydroxylase and 17,20 desmolase.

Significance:

The hormonal control of Leydig cell function and androgen production is an essential component of male sexual development and reproductive capacity. In addition to its significance to the Endocrinology and Reproduction Research program, this project has the potential to provide knowledge of the general actions of peptide and protein hormones upon target cells in tissues other than the gonads.

Proposed Course:

1. (a) Continue investigation on the characteristics of holoenzymes in adrenal and Leydig cell. Autophosphorylation, salt sensitivity, ATP receptors and the modulation of cAMP binding by ATP.

(b) The role of protein kinase in gonadotropin action will be further analyzed by measurement of endogenous protein kinase activity and substrate phosphorylation in dispersed Leydig cells during LH stimulation in vitro.

(c) Continue investigation on the functional compartmentalization of cyclic AMP responses in the Leydig cell. Localization of cyclic AMP bound to receptor protein at different levels of hCG or choleragen stimulation by immunofluorescence microscopy of cyclic AMP in intact Leydig cells.

(d) Role of cyclic nucleotides as mediator of trophic hormone action in the adrenal.

2. Activation of cholesterol side-chain cleavage and cholesterol esterase by LH in purified Leydig cells.

3. The role of co-factors during LH stimulation in the Leydig cell.

Publications:

Podesta, E. J., Dufau, M. L., Solano, A., and Catt, K. J.: Hormonal activation of protein kinase in isolated Leydig cells (electrophoretic analysis of cyclic AMP receptors). J. Biol. Chem. 253: 8994-9001, 1978.

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Sala, G. B., Dufau, M. L. and Catt, K. J.: Gonadotropin action in isolated ovarian luteal cells. Adv. Exp. Med. Biol. 112: 489-497, 1978.

Sala, G. B., Dufau, M. L., and Catt, K. J.: Gonadotropin action in isolated ovarian luteal cells: The intermediate role of adenosine 3'5'-monophosphate in hormonal stimulation of progesterone synthesis. J. Biol. Chem. 254: 2077-2083, 1979.

Sala, G. B., Hayashi, K., Catt, K. J. and Dufau, M. L.: Adrenocorticotropin action in isolated adrenal cells: The intermediate role of adenosine cyclic AMP in stimulation of corticosterone synthesis. J. Biol. Chem. 254: 3861-3865, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00148-04 RR

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Ontogeny of Gonadotropin Receptors and Leydig Cell Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	K. J. Catt	Chief, ERRB	ERRB, NICHD
	M. L. Dufau	Senior Investigator	ERRB, NICHD
Other	V. Chan	Visiting Scientist	ERRB, NICHD
	R. Clayton	Guest Worker	ERRB, NICHD

COOPERATING UNITS (if any)

Department of Medicine, University of Texas Southwestern Medical School, Dallas, Texas (Jean Wilson, M.D., and Fred George, Ph.D.)

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Section on Hormonal Regulation

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is concerned with analysis of developmental characteristics of gonadotropin receptors during fetal life and sexual maturation. Collaborative studies on fetal rabbit testis development in organ culture through the critical period of male sexual development were continued. Cyclic AMP and testosterone responses of normal gonads during this period confirmed that androgen formation by the fetal testis during male sexual differentiation is independent of extrinsic hormone stimulation. The effect of estrogen upon testis function during development was examined in FSH-treated immature hypophysectomized rats. In this model, in which effects via the pituitary are avoided, estrogen was found to significantly decrease LH receptors and testosterone responses, and hypophysectomy was found to increase cyclic AMP production by the Leydig cell. This work provided evidence for a direct inhibitory effect of estrogen on Leydig cell function.

PHS-6040
(Rev. 10-76)

Major Findings:

(A) Fetal Gonads:

Previous studies in progress in the regulation of steroid hormone biosynthesis in fetal rabbit gonads were completed. The results obtained in collaborative studies with J. Wilson and F. George were compared with their finding that the ovary acquires the capacity to convert C19 steroids to estrogens at the same time in development as the enzymatic capacity to form testosterone develops in the fetal testis. However, the absence of LH receptors we observed in the fetal ovary suggests that estrogen production is not subject to hormonal regulation during fetal life. A further aspect of this work was initiated by analysis of protein kinase activity in the fetal testis. Measurement of the enzyme was shown to be possible in the testis of the late-gestation fetus and will be adapted for more detailed analysis of the role of protein kinase in the development of the fetal gonads and the hormonal control of gonadal steroidogenesis during fetal life.

(B) Gonadal Maturation:

The previous finding on the importance of FSH in maintaining LH receptors and testis responses in immature hypophysectomized rats was followed by studies in the role of estrogen in testicular function. In the FSH-treated hypophysectomized immature rat, estrogen was shown to have direct effects on the Leydig cell, including reduction of LH receptors and impairment of testicular androgen responses to exogenous luteotropin. Also, hypophysectomy was found to increase Leydig cell cyclic AMP responses to luteotropin (while reducing androgen responses), suggesting that a pituitary factor regulates the capacity of the Leydig cell to produce cyclic AMP during hormonal stimulation.

Analysis of hormone receptors during development was extended to include those for GnRH in the anterior pituitary and prolactin in the gonads. Methods were established to permit longitudinal analysis of these sites with those for LH and FSH, during maturation of the testis and ovary in the rat.

Significance:

The characterization of pituitary and gonadal receptors and androgen regulation during fetal life and in immature animals will assist in clarification of mechanisms determining male phenotypic sexual development, and those controlling gonadal function during sexual maturation.

Proposed Course:

The role of protein kinase in mediating aspects of fetal testis maturation will be examined, and the development of pituitary function and GnRH receptors will be correlated with the profiles of gonadal receptors for LH/FSH and prolactin, and gonadal steroidogenesis during maturation in the rat.

Publications:

Hsueh, A.J.W., Dufau, M.L., and Catt, K.J.: Direct inhibitory effect of estrogen on Leydig cell function of hypophysectomized rats. Endocrinology 103: 1096-1102, 1978.

George, F.W., Catt, K.J., and Wilson, J.D.: Regulation of the onset of steroid hormone synthesis in fetal gonads. In Hamilton, T.H., Clark, J.H., and Sadler, W.A. (Eds.): Ontogeny of Receptors and Reproductive Hormone Action. New York, Raven Press, 1979, pp. 411-420.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00149-04 RR

PERIOD COVERED
October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Bioassay of Serum Luteinizing Hormone (LH) and Chorionic Gonadotropin

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	Maria L. Dufau	Chief, Section on Molecular Endocrinology	ERRB, NICHD
	Kevin J. Catt	Chief	ERRB, NICHD
Other	Angela Solano	Visiting Fellow	ERRB, NICHD
	Alfonso Garcia-Vela	Guest Worker	ERRB, NICHD
	Gary Hodgen	Chief	PRB, NICHD

COOPERATING UNITS (if any)
Dept. of Gynecology, Harvard Medical School, Mass. General Hospital, Boston, MA; Dept. of Pediatrics, Bay State Medical Center, Springfield, MA; Protein and Polypeptide Hormone Lab., Dept. of Physiology, Univ. of Manitoba, Winnipeg, CAN

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Molecular Endocrinology Section

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Long range studies on the in vitro bioactivity of luteinizing hormone and chorionic gonadotropin in man and other species were continued. The recently developed in vitro bioassay for the measurement of LH and hCG in serum has been more extensively applied to studies on gonadotropin regulation in man and other species.

PHS-6040
(Rev. 10-76)

Objectives:

1. Application of the optimized in vitro bioassay for serum LH measurement of LH in human serum in physiological conditions and pathological states, and during stimulation with luteinizing hormone releasing hormone.

2. Comparative studies with radioimmunoassay, and analysis of bio:immuno (B:I) ratio in normal subjects and pathological conditions.

3. Measurement of biologically active LH in newborn babies from birth to one to two months of age; comparative studies with radioimmunoassay for the intact LH molecule and specific hCG beta-subunit radioimmunoassay.

4. Measurement of bioactive LH in children (boys and girls) of selected age groups through puberty.

5. Measurement of bioactive LH in rhesus monkey in physiological states and during stimulation with LHRH.

6. Development and optimization of the in vitro bioassay for LH in rat pituitary and serum.

7. Physical fractionation of plasma and pituitary extracts from human and animal species with subsequent analysis of biological active species by RICT assays.

8. Development of new assays to assess biological activity of gonadotropic hormones.

Methods Employed:

1. In vitro bioassay for serum gonadotropin based on testosterone production in collagenase-dispersed rat interstitial cells (RICT assay).

2. Radioimmunoassays for human LH and heterologous radioimmunoassay for monkey monkey gonadotropin. Radioimmunoassay for rat LH and for rLH subunits.

Major Findings:

1. In vitro Assay of LH in Rat Pituitary and Serum. Modulation of LH Bioactivity by Gonadal Steroids.

The frequent use of the rat as an animal model in reproductive endocrinology made the bioassay of rat serum LH an obvious application for the RICT assay. In particular, the use of rats for analysis of LH bioactivity of stored secreted hormone and the effect of steroids on gonadotropin modulation would be an advantage for the understanding of noted bio:immuno differences observed in human subjects.

Precise measurements of bioactive and radioimmunoactive levels of plasma LH in male and female rats can be performed by a modified RICT and RIA

procedures. Modifications were necessary to improve the sensitivity of both assays to permit detection of low LH levels circulating in the rat. The serum LH B:I (bioimmuno) ratio in male rats was 1.2, significantly higher than in pituitary extracts 0.97. During the estrous cycle an increase in serum LH B:I ratio was observed at the time of the LH surge, with simultaneous decrease of pituitary B:I ratio. This indicated that the released hormone was more active than the stored hormone. After castration the B:I ratio was reduced to 50% of control values, this was more pronounced in serum than in pituitary. The decrease of B:I ratio of LH in castrated males was not due to increased subunit production and was prevented by administration of testosterone propionate. By these results it is concluded that androgen or their metabolites can modify the bioactivity of the circulating and stored forms of the LH molecule.

2. Bioactive and Immunoreactive Serum LH in Normal Prepubertal and Pubertal Boys.

Qualitative and quantitative changes in the pattern of secretion of immunoreactive LH and FSH occur at pubescence. Maturational change in mean levels of bioassayable LH has been demonstrated. Prepubertal boys did not have detectable (<1 mIU/ml) concentrations of bioactive LH, while mean levels of bioactive LH were nearly 14 mIU/ml in pubertal subjects. In contrast, immunoreactive LH was detectable in 6 of 12 prepubertal boys, probably due to the relative insensitivity of the RIA.

The very low levels of bioactive LH in the prepubertal boys are similar to those reported in the prepubertal rhesus monkey. Significant increments of bioactive LH were detected between prepubertal and early pubertal years; during the same period, immunoactive LH rose minimally or not at all. These studies demonstrating bioassayable LH in young children confirm earlier studies of urinary gonadotropins which were quantified by the mouse uterine weight bioassay.

The data indicate that biologically active LH is low in the serum of prepubertal subjects and increases as the child enters puberty. There is little quantitative relationship between bioactive and immunoactive LH. The nature of the structural or conformational form of LH which differs with the onset of puberty remains to be elucidated. Recent studies in the rat, however, have demonstrated that LH bioactivity can be modulated by gonadal steroids.

The ratio of bioactive to immunoreactive LH (B/I ratio) in several clinical conditions may be contrasted with the present data. In the prepubertal boys, the B/I ratio of less than 0.6 was lower than the adult female ratio of about 1, but the proximity to the sensitivity limits of both bio and immuno assays must be recognized. In contrast, pubertal males had a mean B/I ratio of approximately 2.1 comparable to normal adult males and to girls with Turner's syndrome.

3. Demonstration of Potent, Gonadotropin-Like Biological Activity in the Serum of Rats During Midpregnancy.

Hormonal maintenance of the rat corpus luteum during midpregnancy has remained an enigma for nearly 50 years. Although the ovary is indispensable

for early pregnancy maintenance, its tropic support is shifting from the pituitary to the placenta between days 8 and 11 of pregnancy. Whereas hypophysectomy before day 11 of pregnancy results in abortion, pituitary PRL is not an essential progestational agent after day 8. Moreover, it has been suggested that LH might be an important stimulus to the rat ovary between days 8 and 11. However, not only are radioimmunoactive serum levels of LH relatively low throughout pregnancy but also the conceptus is probably required for LH to exert its full luteotropic effect. Furthermore, the biological activity of the known circulating hormones cannot fully account for the potent luteotropic properties of midpregnancy serum or for the early increase in intraluteal estrogens which synergize with rat placental lactogen to support pregnancy after day 12.

The gonadotropin-like activity (GnLA) of serum from pregnant rats was measured using the rat interstitial cell testosterone (RICT) bioassay. Serum GnLA was elevated on day 9 of pregnancy, peaked at 7.2 ug rat LH-RP1 equivalents/ml on day 11 and declined to undetectable levels by day 15. Serum LH, measured by homologous RIA, was consistently low (<20 ng/ml) during pregnancy, except near term. Rat placental lactogen (rPL), which was measured in the same serum samples by rat ovary radioreceptor assay (RRA), reached maximal concentrations on days 12 and 13 of pregnancy.

These data suggest the presence in pregnancy serum of a potent-gonadotropin-line hormone, different from pituitary LH, whose origin is unknown. Furthermore, there are discrepancies between the times of appearance of this GnLa and rPL.

Significance to Biomedical Research:

The application of the RICT assay to the measurement of LH in human serum during LHRH stimulation has provided information about the qualitative and quantitative changes in the LH molecule at different stages of the menstrual cycle. Also, the application of the RICT assay to the measurement of LH in monkey serum has provided the scientific community with a convenient method for the measurement of LH in monkey serum has provided the scientific community with a convenient method for the measurement of this hormone in serum. The recent development in the increasing sensitivity of the RICT assay (5- to 10-fold), is of great value for the measurement of bioactive LH levels in children and in adults during suppression therapy. Also this modification permitted measurement of circulating LH in rats.

The RICT assay can be applied to different laboratories all over the world, and is expected to give similar results when identical standards are employed. This is an improvement over certain aspects of RIA which are subjected to the individual characteristics of each antibody employed for assay.

The high sensitivity of the RICT assay will permit measurement of the levels of LH in serum from birth to puberty with good precision. This will also offer advantages over the RIA, which at these early times frequently measures levels just slightly above the lower limit of the sensitivity of the assay.

Proposed Course:

1. Further investigation to gain insight into the nature of the B:I ratio differences observed between the different groups of subjects will include: (a) examination of different LH pools released from the pituitary during stimulation studies; (b) physical characterization of serum LH of the different groups of subjects.

2. Evaluation of LH values from birth through puberty.

3. Studies on the measurement of bioactive LH in serum of rhesus monkey during LHRH stimulation.

4. Studies on the modulation of rat LH bioactivity by steroids and on the biosynthesis of LH in the pituitary will be continued.

Publications:

Solano, A. R., Dufau, M. L. and Catt, K. J.: Bioassay and radioimmunoassay of serum luteinizing hormone in the male rat. Endocrinology 105: 372-381, 1979.

Beitins, I. Z., Dufau, M. L., O'Laughlin, Bercu, B., McArthur, J. W., Crawford, J. D. and Catt, K. J.: Comparison of biological and immunological activities in serum LH in normal women during LHRH infusion and patients with Turner's syndrome treated with estrogen. Symposium of LHRH. December 9-10, 1977, Memphis Tennessee. In press.

Hodgen, G. D., Chen, H-C., Dufau, M. L., Klein, T. A. and Michelle, D. R.: Transitory hCG-like activity in the urine of some IUD users. J. Clin. Endocrinol. Metab. 46: 698-701, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00150-04 RR

PERIOD COVERED
October 1, 1978 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Characterization and Purification of LH/hCG Receptors and Adenylate Cyclase.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	M. L. Dufau	Chief, Section on Molecular Endocrinology	ERRB, NICHD
	K. J. Catt	Chief	ERRB, NICHD
Other	J. Wimalasena	Visiting Fellow	ERRB, NICHD
	K. Hayashi	Visiting Fellow	ERRB, NICHD

COOPERATING UNITS (if any)

None

LAB/BRANCH
Endocrinology and Reproduction Research Branch, NICHD

SECTION
Section on Molecular Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This is a long-term project on the solubilization, characterization and purification of LH/hCG and FSH receptors and adenylate cyclase of testis and ovary. In addition to analyzing the properties of gonadotropin receptors and adenylate cyclase, these studies will lead to an analysis of the physical and functional relationships of the binding site and the enzyme complex in the cell membrane and in solution.

PHS-6040
(Rev. 10-76)

Objectives:

1. Characterization and Purification of LH/hCG Receptors and Adenylate Cyclase.

a) Analysis of the physical and functional relationships of the LH/hCG binding site and the enzyme complex in the cell membrane.

Previous studies from our laboratory have indicated that gonadotropin receptor and adenylate cyclase are physically separate entities that can be resolved during appropriate fractionation procedures. However these findings did not exclude the possibility that the two species are associated in some manner in the lipid bilayer of the plasma membrane. It was therefore of interest to follow the functional properties of LH receptors from gonadal tissue after incorporation into heterologous steroidogenic cells. Studies on the uptake of ovarian LH receptors by adrenal cells showed that these receptors were incorporated into the cell membrane and were subsequently available to bind ^{125}I-hCG. The incorporated receptors have been shown to be functionally coupled to adenylate cyclase as demonstrated by cAMP and corticosterone responses to gonadotropin during in vitro stimulation of adrenal cells. It can be concluded from these studies that gonadotropin receptors and adenylate cyclase exist in the cell membrane as separate entities that become associated to form an active complex during receptor occupancy by the homologous hormone.

2. Solubilization and characterization of gonadotropin receptors of testis and ovary.

Soluble ovarian LH receptors have been prepared by extraction with nonionic detergents, and by association with ovarian lipids during homogenization in the absence of detergents. In addition to these previously described forms of the ovarian LH receptors, we have now characterized a group of water soluble LH receptors from pseudopregnant rat ovaries. We have demonstrated that high speed supernatants of aqueous or low ionic strength buffer extracts of the rat ovary contain soluble LH receptors. These receptors are proteins with high affinity and specificity for LH-like gonadotropins. These receptors are stable and retain 60% to 80% of their binding capacity after lyophylization. Fractionation of soluble receptors by gel filtration and gel electrophoresis demonstrated four binding species with MWs of 160,000, 48,000, 24,000, and 9000. These findings demonstrate that the specific binding site for LH is present and active in proteins much smaller than the 6.5S receptor extracted by nonionic detergents. The stability of these LH binding sites is of value for further purification and analysis of structural determinants of gonadotropin binding.

Proposed Course:

Purification of LH receptors and adenylate cyclase from gonadal cell membranes will be pursued, to permit further characterization of the purified receptors and their functional relationship to adenylate cyclase.

Significance:

The characterization of LH receptors and their relationships to adenylate cyclase will assist in understanding the basic biochemical mechanisms which control the reproductive functions of the testis and ovary. Also, further understanding of the receptor-cyclase interaction will be of general value in understanding the mechanisms of action of protein hormones.

Publications:

Dufau, M. L., Hayashi, K., Sala, G., Baukal, A. and Catt, K. J.: Gonadal luteinizing hormone (LH) receptors and adenylate cyclase: Transfer of functional ovarian LH receptors to adrenal fasciculata cells. Proc. Natl. Acad. Sci., USA 75: 4769-4779, 1978.

Dufau, M. L. and Catt, K. J.: Gonadotropin receptors and regulation of steroidogenesis in testis and ovary. In Munson P., Diczfaluzy, E. (Eds.): Vitamins and Hormones, Academic Press, New York, Vol 36: 1978, pp. 461-600.

Dufau, M. L., Hayashi, K., Sala, G. and Catt, K. J.: Gonadal LH receptors: Resolution from adenylate cyclase and transfer to heterologous cells. In Marsh, J., Channing, C. and Sadler, W. (Eds.): Ovarian Symposium, Miami, Plenum Press, New York, March 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00151-04 RR

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Regulation of Gonadotropin Receptors in Testis and Ovary

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	K. J. Catt	Chief	ERRB, NICHD
	M. L. Dufau	Chief, Section on Molecular Endocrinology	ERRB, NICHD
Other	J. Harwood	Senior Staff Fellow	ERRB, NICHD
	T. Davies	Guest Worker (USPHS Fellow)	ERRB, NICHD
	V. Chan	Visiting Scientist	ERRB, NICHD
	T. Chen	Staff Fellow	ERRB, NICHD
	M. Katz	Clinical Associate	ERRB, NICHD
	R. Clayton	Guest Worker (MRC Fellow)	ERRB, NICHD
	M. Katikineni	Research Associate	ERRB, NICHD

COOPERATING UNITS (if any)

None

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Section on Hormonal Regulation

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	4.0	1.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This program is concerned with analysis of peptide hormone receptors in testis and ovary, with particular reference to the control of receptor concentration by changes in homologous and heterologous hormones. The recently-described desensitization of adenylate cyclase and steroidogenic responses to gonadotropic hormones is followed by receptor loss, by turnover or processing of the hormone-receptor complex. These processes were further investigated in Leydig cells and ovarian luteal cells to determine the effects of hormone-induced receptor regulation on the responsiveness of the endocrine target cells, and to analyze the mechanism and consequences of target-cell desensitization.

PHS-6040
(Rev. 10-76)

Objectives:

To analyze the regulation of gonadotropin receptor biosynthesis, expression and turnover in gonadal target cells.

Methods Employed:

Receptor concentration and affinity were measured by binding studies with ^{125}I-labeled LH, hCG, hFSH and hGH (for prolactin receptors), and [^{3}H]dihydroalprenolol for β-adrenergic receptors. Analysis of binding data was performed by computer methods applied to saturation curves and Scatchard plots. Luteinized ovaries were induced in immature female rats by treatment with PMSG and hCG. Isolated luteal and Leydig cells were prepared from ovary and testis, by collagenase dispersion and purification by density gradient centrifugation. Receptor binding (to LH, FSH, PRL and and β-receptors) was measured in tissue homogenates and whole cells. Adenylate cyclase was measured in particulate ovarian fractions, and cyclic AMP and steroid production were determined in dispersed luteal and Leydig cells by radioimmunoassay.

Major Findings:

1. Regulation of testicular LH, FSH and PRL receptors.

Regulation of LH receptors in the testis is highly sensitive to the circulating LH concentration, and marked loss of receptor sites has been demonstrated after treatment with exogenous gonadotropins such as hCG and ovine LH. Treatment with potent LHRH analogs has also been shown to cause loss of testicular LH receptors in rats, presumably due to elevation of endogenous gonadotropin secretion. To analyze the relationship between the changes in plasma gonadotropins and the receptor content of the testis, the effects of single doses (0.1, 1, and 10 μg) of the natural LHRH decapeptide on LH secretion and testicular receptors were examined in 55-day-old rats.

After sc administration of LHRH, serum LH and testosterone values peaked at 15 and 60 min, respectively, and declined to normal values over the subsequent 4 h. In contrast with these rapid and transient plasma hormone responses, the LH and PRL receptor content of the testis exhibited delayed and protracted changes after single doses of LHRH. Between 8-48 h after administration of LHRH, there was a marked and consecutive loss of PRL-, and LH-binding sites, each of which returned to control values after about 5 days. The magnitude and duration of receptor loss was related to the dose of LHRH administered, with almost total abolition of binding sites after treatment with 10 μg LHRH.

The time sequence of plasma gonadotropin levels in relation to LH receptor regulation was similar to that caused by administration of exogenous gonadotropin, suggesting that the transient peak in serum LH initiated a process of negative regulation that was manifested by the subsequent decrease in testicular receptor sites. The marked changes in gonadotropin and PRL receptors probably contribute to the inhibitory effects of LHRH treatment on ovarian and testicular function. In addition, these actions of LHRH can be utilized to

investigate the complex process of multiple receptor loss and regeneration that may accompany the physiological actions of pituitary hormones in the testis.

2. Regulation of gonadotropin and β-adrenergic receptors in ovarian luteal cells.

The actions of epinephrine on luteal cell functions, and the effects of gonadotropin-induced luteal desensitization upon β-adrenergic responses, were examined in the luteinized rat ovary. Both epinephrine and LH stimulated adenylate cyclase activity in ovarian homogenates, and increased the production of cyclic AMP and progesterone in luteal cells. Maximally effective doses of the two hormones were not additive upon adenylate cyclase activity and steroidogenesis. The actions of epinephrine on adenylate cyclase and progesterone production were inhibited by propranolol but not by phentolamine, and neither adrenergic antagonist influenced the actions of LH. These results indicate that LH and β-adrenergic effectors act through independent receptors on a common pool of adenylate cyclase to stimulate progesterone production in the luteal cell.

In ovaries from rats treated with desensitizing doses of hCG, the decreased ability of LH/hCG to stimulate adenylate cyclase activity was accompanied by a transient loss of the enzyme response to epinephrine. In partially desensitized ovaries, there was no change in the half-maximal dose for epinephrine stimulation of adenylate cyclase activity. The loss of enzyme responsiveness to LH/hCG and epinephrine was proportional to the desensitizing dose of hCG. In luteal cells prepared from desensitized tissues, the impaired response of progesterone production to LH was accompanied by a decreased response to epinephrine. The production of cyclic AMP by isolated luteal cells was correspondingly decreased in response to both ligands. The number of LH receptors was also markedly reduced, but no significant change in the number of β-adrenergic receptors was detected by assay with [^{3}H]dihydroalprenolol.

These findings indicate that LH receptor-mediated desensitization of the ovarian luteal cell by hCG leads to loss of the response to epinephrine, as well as the characteristic responses to LH. Since the two hormones act via independent receptors to evoke cyclic AMP production and steroidogenesis, it is proposed that desensitization by gonadotropin leads to a defect in the coupling of β-adrenergic receptors to adenylate cyclase.

3. Specificity of desensitization by gonadotropin.

Adenylate cyclase activity of luteinized ovaries from gonadotropin-primed immature rats was stimulated by luteinizing hormone (LH), epinephrine and prostaglandin E_1. Treatment of primed rats with human chorionic gonadotropin (hCG) caused a time-dependent loss of the adenylate cyclase response to LH, and the enzyme also became refractory to stimulation by epinephrine. Conversely, treatment of primed rats with epinephrine caused a time-dependent loss of the adenylate cyclase response to epinephrine, but the enzyme remained responsive to LH. The refractoriness of adenylate cyclase to epinephrine after

desensitization by hCG or epinephrine was not reversed by GPP(NH)p. These studies show that "cross-desensitization" of epinephrine-responsive adenylate cyclase activity in the ovary is induced by LH-receptor interaction, whereas β-adrenergic desensitization causes more specific refractoriness to epinephrine with no change in the LH receptor activation mechanism. This suggests the existence of fundamental differences in mechanisms of adenylate cyclase activation and/or desensitization by catecholamines and glycoprotein hormones.

Significance:

These findings extend knowledge of the regulation of receptor content and receptor-mediated processes in peptide hormone target tissues. The results are of general application to protein hormone receptors, and of particular relevance to the control of gonadal cell function and reproductive activity.

Proposed Course:

The nature of the processes concerned in receptor synthesis and turnover will be further analyzed in both testis and ovarian cells. Endocytosis of hormone-receptor complexes will be analyzed in relation to the processes of cell activation that depend upon peptide hormone stimulation. The basis for the multiple receptor loss caused by LHRH will be further investigated, and causes sought for the regression in testis function caused by LHRH derivatives. The direct effects of LHRH on the gonads will also be analyzed in more detail.

Publications:

Conn, P.M., Conti, M., Harwood, J.P., Dufau, M.L., and Catt, K.J.: Internalisation of gonadotrophin-receptor complex in ovarian luteal cells. Nature 274: 598-600, 1978.

Harwood, J.P., Conti, M., Conn, P.M., Dufau, M.L., and Catt, K.J.: Receptor regulation and target cell responses: Studies in the ovarian luteal cell. Mol. Cell. Endocrinol. 11: 121-135, 1978.

Catt, K.J., Baukal, A.J., Davies, T.F., and Dufau, M.L.: Luteinizing hormone releasing hormone-induced regulation of gonadotropin and prolactin receptors in the rat testis. Endocrinology 104: 17-25, 1979.

Catt, K.J., Harwood, J.P., Aguilera, G., and Dufau, M.L.: Hormonal regulation of peptide receptors and target cell responses. Nature 280: 109-116, 1979.

Catt, K.J., Harwood, J.P., Richert, N.D., Conn, P.M., Conti, M., and Dufau, M.L.: Luteal desensitization: Hormonal regulation of LH receptors, adenylate cyclase and steroidogenic responses in the luteal cell. In Channing, C.P., Marsh, J.M., and Sadler, W.A. (Eds.): Ovarian Follicular and Corpus Luteum Function. New York, Plenum Press, 1979, pp. 647-662.

Harwood, J.P., Dufau, M.L., and Catt, K.J.: Differing specificities in the desensitization of ovarian adenylate cyclase by epinephrine and human chorionic gonadotropin. Mol. Pharmacol. 15: 439-444, 1979.

Davies, T.F., Katikineni, M., Chan, V., Harwood, J.P., Dufau, M.L., and Catt, K.J.: Lactogenic receptor loss in hormone-stimulated steroidogenic cells. Nature, 1979, in press.

Clayton, R.N., Harwood, J.P., and Catt, K.J.: Gonadotropin-releasing hormone analogue binds to luteal cells and inhibits progesterone production. Nature, 1979, in press.

Davies, T.F., Hodgen, G.D., Dufau, M.L., and Catt, K.J.: Regulation of primate testicular luteinizing hormone receptors and steroidogenesis. J. Clin. Invest., 1979, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00152-04 RR

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Characterization and radioligand assay of gonadotropin receptors.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	K. J. Catt	Chief	ERRB, NICHD
	M. L. Dufau	Senior Investigator	ERRB, NICHD
Other	T. F. Davies	USPHS Fellow	ERRB, NICHD

COOPERATING UNITS (if any)

P. C. Walsh, Dept. of Urology, Johns Hopkins School of Medicine
G. D. Hodgen, Pregnancy Research Branch, NICHD

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Section on Hormonal Regulation

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is concerned with the development and use of analytical procedures for identification and characterization of gonadotropin receptor sites in the testis and ovary. Methods of gonadotropin labeling, characterization of the labeled tracer, and optimization of receptor assay methods, are evaluated and improved. Computerized methods for analysis of binding data from kinetic and equilibrium experiments, plus corrections for the tracer characteristics, are applied to studies on the properties of gonadotropin receptors. Natives and modified gonadotropins are evaluated for receptor binding activity, and receptor characteristics are analyzed in animal and human tissues. Current studies include characterization of the human testicular receptors for LH and FSH.

PHS-6040
(Rev. 10-76)

Objectives:

To characterize the binding properties of particulate gonadotropin receptors in gonadal cells, homogenates and membrane preparations. Also, to develop improved radioligand-receptor assays for LH/hCG and FSH, to apply such methods to assay of gonadotropin activity in vitro and structure-function studies on chemically modified gonadotropins, and to analyze the species specificity of gonadotropin receptors.

Methods Employed:

Quantitative binding studies with labeled gonadotropins. Radioligand-receptor assays of gonadotropins. Affinity chromatography upon receptor sites. Computer analysis and curve-fitting of receptor binding data. In vitro bioassay of LH in dispersed Leydig cells.

Major Findings:

The interaction of [^{125}I]hCG with the primate LH receptor was investigated in particulate fractions and cell suspensions from human and rhesus testicular tissue. Binding to testis homogenates was saturable, dose and time dependent, and displaced by low concentrations of unlabeled human LH and hCG. Both the human and rhesus LH receptors were of high affinity (K_a = 2-3 x 10^{10} M^{-1}) and low capacity (23-146 fmol/g), and both failed to cross-react with heterologous LH at concentrations up to 1 ug/ml. In contrast, the rat testis LH receptor interacted with several heterologous LH preparations in nanogram concentrations. The species specificity of the primate LH receptor was also demonstrated by recombination studies with isolated subunits of porcine LH and hCG. The presence of the -subunit of hCG, irrespective of the recombined -subunit, conferred the ability to inhibit binding of [^{125}I]hCG by the primate testis homogenates. The porcine -subunit, when recombined with the -subunit of hCG, failed to inhibit binding of [^{125}I]hCG to primate testicular receptors, whereas the recombined preparation was active in binding to rat testis. Collagenase-dispersed human and rhesus interstitial cells responded with increased testosterone production when stimulated by homologous gonadotropins in vitro. Such primate interstitial cells were sensitive to low concentrations of LH and hCG and exhibited the same species specificity as the receptor-binding studies performed with testis homogenate.

These studies have shown that LH receptors in the primate testis possess marked species specificity for gonadotropins with interstitial cell-stimulating activity. The species specificity of the primate LH receptor was shown to depend upon recognition of a conformation endowed by the -subunit of the human glycoprotein hormone. Enzyme-dispersed interstitial cell preparations retained biological responses to human gonadotropins and provided a simple and sensitive in vitro primate bioassay for gonadotropic peptides. The similar properties of human and rhesus testis homogenates and interstitial cell fractions indicate that rhesus testes provide a useful model for research into human gonadotropin receptors and their regulation.

Significance:

Radioligand-receptor systems continue to be of considerable value for hormone assay, and for structure-function studies on hormone molecules and receptor sites. The high species specificity of the primate LH receptor provides an explanation for the poor responsiveness of human gonads to lutotropins from lower animals.

Proposed Course:

Studies on the temperature-dependence and thermodynamic properties of hormone-receptor interactions will be pursued. Also, radioligand-receptor assays will be compared with in vitro bioassay during analysis of the biological activities of native and modified gonadotropins. The basis of the species specificity of the primate LH receptor will be investigated, and characterization of the primate FSH receptor will be performed.

Publications:

Davies, T. F., Dufau, M. L. and Catt, K. J.: Gonadotrophin receptors: characteristics and clinical applications. Clinics in Obstetrics and Gynecology, 5:329-362, 1978.

Catt, K. J. and Dufau, M. L.: Gonadotropin receptors and regulation of interstitial cell function in the testis. In O'Malley, B. and Birnbaumer, L. (Eds.): Receptors and Hormone Action. New York, Academic Press, 1978, Vol. III, pp. 291-338.

Davies, T. F., Walsh, P. C., Hodgen, G. D., Dufau, M. L., and Catt, K. J.: Characterization of the primate lutenizing hormone receptor in testis homogenates and Leydig cells. J. Clin. Endocrinol. Metab. 48:680, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00157-04 RR

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)
Physiology of Puberty

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	D. Lynn Loriaux, M.D., Ph.D., Head	DES, ERRB, NICHD, NIH
Other	G. Cutler, M.D., Clinical Associate	ERRB, NICHD, NIH
	R. Schiebinger, M.D., Clinical Associate	ERRB, NICHD, NIH
	B. Albertson, HEW Post Doctoral Fellow	ERRB, NICHD, NIH
	F. Cassorla, Clinical Associate	PNMB, NICHD, NIH

COOPERATING UNITS (if any)

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Developmental Endocrinology Section

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to gain insight into the physiologic processes underlying normal and abnormal pubertal progression. Principal areas of investigation include the mechanisms of adrenarche and the temporal changes in hypothalamic function with respect to gonadotropin regulation.

PHS-6040
(Rev. 10-76)

Progress:

1. Satisfactory assays for 17 and 21 hydroxylase, 17-20 demolase and Δ 4-5 isomerase - 3 β hydroxy steroid dehydrogenase have been established for adrenal microsomes.

2. These assays have been applied to dog and rabbit adrenal microsomes over the period of pubertal maturation. No change was observed in rabbit enzyme activities. The dog revealed a marked increase in 17-hydroxylase. This was accompanied by an increase in plasma 17-hydroxy progesterone.

Significance:

The only known model for human adrenarche is the chimpanzee. The above findings coupled with the histologic changes seen in the dog adrenal suggestive of a developing zona reticulars imply that the dog might serve as a marker for the process in this species.

Proposed Course:

1) The investigation of the role of the hypothálamic-pituitary system in the induction of adrenarche in the chimpanzee. This study employs human pituitary extract as the test substance and is being done in collaboration with the Primate research facility at Holloman Air Force Base, Alamagordo, New Mexico.

2) The identification of the enzyme changes that occur in the adrenal gland during adrenarche. Assays for 17-hydroxylase, 17-20 desmolase, 21-hydroxylase, and 3 β-hydroxysteroid dehydrogenase- Δ 4-5 isomerase will subsequently be applied to chimpanzee and human adrenal specimens.

3) The investigation of the role of the human pituitary in the maintenance of adrenal androgen secretion in man. This study is being performed in collaboration with the neurosurgery division of Walter Reed Army Hospital.

4) The investigation of the role of prolactin in adrenal androgen secretion. DHA sulfate production rates are being examined before and during bromocryptine therapy and before and after hypophysectomy in an attempt to quantitate the influence of prolactin on adrenal androgen secretion.

Publications:

Cutler, G.B., M. Glenn, G. Hodgen, C. Graham, and D.L. Loriaux: Adrenarche: A Survey of Rodents, Domestic Animals, and Primates. Endocrinology, 103: 2112, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00158-04 RR

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Pathophysiology of Gynecomastia and Hirsutism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	D. Lynn Loriaux, M.D., Ph.D., Head, Endocrine Service Unit,	ERRB, NICHD, NIH
Other	G. Cutler, Jr., M.D., Medical Officer	ERRB, NICHD, NIH
	C. Eil, M.D., Clinical Associate	ERRB, NICHD, NIH
	S.E. Davis, M.D., Senior Investigator	ERRB, NICHD, NIH
	M.B. Lipsett, M.D., Director, Clinical Center	
	S. Kono, Visiting Scientist, Clinical Center	

COOPERATING UNITS (if any)

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Developmental Endocrinology Section

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to provide information for the understanding of disorder phenotypic secondary sexual expression, including such disorders as gynecomastia and hirsutism. The current principal area of investigation is end organ steroid-receptor interaction.

PHS-6040
(Rev. 10-76)

Progress:

1. Gynecomastia

Using a newly developed assay for androgen receptors in cultured human fibroblasts, it has been shown that androgen receptor concentrations in fibroblasts from genital and areolar skin is the same in young normal men and age matched patients with gynecomastia.

2. Hirsutism:

Current evidence tends to support the hypothesis that virtually all cases of idiopathic hirsutism can be explained by abnormal ovarian secretion of androgen. We have made a detailed study of the adrenal secretion of the known androgens and their prohormones using ACTH stimulation as a probe to amplify inapparent adrenal abnormalities. We have found that roughly half of patients so tested exhibit abnormalities of adrenal androgen secretion. Some of these have been compatible with known enzymatic deficiencies, such as 21-hydroxylase deficiency and Δ 4-5 isomerase 3β - hydroxy steroid dehydrogenase deficiency. Others conform to no theoretically plausible pattern.

Significance:

1. Gynecomastia:

Our current hypothesis explaining idiopathic gynecomastia is that it represents a form of local androgen resistance. The finding that androgen receptors are normal in this condition is evidence against that hypothesis.

2. Hirsutism:

The finding of a high incidence of adrenal steroidogenic abnormalities in patients with idiopathic hirsutism suggests that the adrenal may play a more important role in the etiology of this disorder than is currently believed. If this can be documented, a rational therapy, exogenous glucocorticoid, would exist for these patients. This would also explain why both gonadotropin suppression and adrenal suppression, when used alone, are only effective in about 50% of patients.

Proposed Course:

1. Gynecomastia

The ability of fibroblasts and lipocytes to aromatize androstenedione and testosterone will next be examined. Fibroblast aromatization will be compared in normals and patients with gynecomastia. Lipocyte aromatization will be compared between breast specimens with and without microscopically apparent ductular hypectrophy.

Publications:

1. Marynick, S.P., B.C. Nisula, J.C. Pita, and D.L. Loriaux: Persistant Pubertal Macromastia. J. Clin. Endocr. Metab., in press.

2. Cutler, Gordon B., Jr., Mark A. Sauer, and D.L. Loriaux: SC25152: A Potent Mineralocorticoid Antagonist with Decreased Antiandrogenic Activity Relative to Spironolactone. J. Pharm. Exp. Ther., 209: 144, 1979.

3. Braunstein, G.D., and D.L. Loriaux: Hypogonadism in Chronically Lead-poisoned Men. Infertility, 1: 33, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00160-04 RR

PERIOD COVERED

October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Biology of the Adrenocortical Cell: Molecular Events in Steroid Biosynthesis and Secretion

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	Charles A. Strott	Senior Investigator	ERRB, NICHD
Other	Prabhati Ray	Visiting Fellow	ERRB, NICHD
	Alan Goff	Visiting Fellow	ERRB, NICHD

COOPERATING UNITS (if any)

None

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	3	1

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long-term aim of this project is to explore in greater depth, the Biology of the steroid-synthesizing cell. There are several areas of uncertainty as to the molecular events involving 1) initiation, propagation, and termination of steroid synthesis, 2) intercompartmental movement of steroid precursors and intermediates, 3) secretion of steroid products. Other areas of uncertainty involve 1) the interaction of subcellular organelles, e.g., mitochondria, endoplasmic reticulum, Golgi apparata, microtubules, microfilaments, lipid droplets, etc. 2) cell-cell interaction or zone-zone interaction (different cells are programmed to secrete predominantly one type of steroid, i.e., glucocorticoid, mineralocorticoid, and sex steroids), 3) life-span and turnover of adrenocortical cells. Current areas of research involving: 1) identification, characterization, and physiology of noncatalytic proteins (membrane bound and/or soluble) of the adrenal cortex that interact with specific steroid ligands; 2) examination of the role of microtubules and contractile proteins in steroid synthesis; 3) identification, characterization and physiology of adrenocortical secretory proteins; 4) examination of soluble stimulatory and inhibitory factors.

PHS-6040
(Rev. 10-76)

Project Description

I. Non-catalytic steroid-binding proteins.

A. Rationale: The rate-limiting step in steroid synthesis in considered to be the conversion of cholesterol to pregnenolone (i.e., the cholesterol lyase reaction). This reaction occurs at an intramitochondrial site (possibly associated with the outer membrane). Cholesterol, which is an absolute requirement for steroid synthesis, is a ubiquitous sterol, being found in membranes and stored esterified to fatty acids in cytoplasmic lipid droplets; it can also be made available through de novo synthesis and extraction from the circulation. The exact location and pool size of cholesterol utilized in steroid synthesis are not known. It is presumed, however, that the principal source of cholesterol is extramitochondrial. If this is true, then there must be a mechanism for transporting cholesterol into the mitochondrion where the cholesterol lyase is located. The molecular events involved in this transport are not known. Similarly, pregnenolone, the product of the rate-limiting step must move to specific extramitochondrial sites for further enzymatic alterations leading to the formation of cortisol, aldosterone, or one of the sex steroids. The molecular events involved in this transport are also not known. It is conceivable that specific proteins acting as transport vehicles are involved.

B. Major Findings: Using standard techniques of subcellular fractionation and methods of protein separation, six specific proteins have been identified to date.

1. Cholesterol/cholesterol sulfate-binding protein No. 1. This is a heat stable protein which has been partially characterized. The initial characterization studies have been published in the Journal of Steroid Biochemistry, 9:721-730, 1978.

2. Cholesterol/cholesterol sulfate-binding protein No. 2. This is a heat sensitive protein whose initial characterization is in press in Journal Steroid Biochemistry, 1979. Purification of these two proteins is now being undertaken.

3. 20 α-hydroxycholesterol-binding protein. The studies here are still preliminary.

4. Pregnenolone-binding protein. This is a heat labile protein whose initial characterization has been published in the Journal of Biological Chemistry, 252: 464-470, 1977. Purification of this protein is currently being completed.

5. Pregnenolone sulfate-binding protein. This is a heat labile protein (clearly distinct from the pregnenolone-binding protein) whose initial characterization was published in Biochemistry, 17: 4557-4563, 1978.

6. 21-hydroxypregnenolone-binding protein. This is a heat labile protein whose initial characterization is currently being completed.

C. Significance: The physiological importance of these findings remains to be determined. From a theoretical point of view, however, they are quite interesting. It is well recognized that labile and stable protein factors play a role in control and regulation of steroid synthesis but to date, no specific proteins have been described. Our studies represent the first such undertaking of this kind. Much needs to be done yet, but given the fact that heat-labile and heat activated proteins which bind specifically cholesterol and cholesterol sulfate, substrates for the side-chain cleavage enzyme system, and heat-labile proteins which bind specifically pregnenolone and pregnenolone sulfate, products of the side-chain cleavage enzyme system, and the fact that the cleavage enzyme system (cholesterol lyase) is considered the rate-limiting step in steroid synthesis, these findings cannot be overlooked and are of potential importance. In addition, there may be other noncatalytic proteins involved in other steroid enzyme systems of the adrenal cortex which when absent could be pathologically related to certain well-recognized enzyme deficiency diseases of the adrenal cortex, viz, congenital adrenal hyperplasia.

D. Current and Future Plans: (1) To continue characterizing the proteins identified to date. (2) To continue our search for other noncatalytic proteins. (3) To complete purification of these proteins using a variety of techniques. (4) To use highly purified preparations to a) examine intrinsic enzymatic activity, and b) to prepare antibodies for immunological studies. (5) To reconstitute the cholesterol lyase system and other steroid mixed function oxidase systems. (6) To examine the subcellular localization of these proteins by tagging experiments. (7) To examine regulation of their synthesis and metabolism.

II. Microtubules.

A. Rationale: Enzyme systems involved in steroid biosynthesis are compartmentalized (mitochondrial, micorsomal,? soluble) but nothing is known about the molecular mechanisms of transfer of steroids from compartment to compartment nor is anything known about the molecular mechanisms involved in steroid secretion. It has been proposed that microtubules or microfilaments may function in this capacity and thus studies have been undertaken to examine this possibility.

B. Major Findings: (1) Our initial efforts were to establish methods for qualitative analysis. A study has been completed comparing colchicane-binding activity (tubulin) in the adrenal cortex of dog, monkey, pig, guinea pig and cow, and brain of the pig and guinea pig. In addition, electron microscopic studies of adrenocortical microtubules have been made. A portion of these studies have been published in Biochimica et Biophysica Acta 495: 119, 1977. (2) There is some inconsistency in the literature concerning the effect of antimicrotubular drugs on adrenal steroidogenesis.

One report indicated that these drugs stimulated steroidogenesis in a mouse tumor cell line, while another report found no such stimulation using normal rat adrenocortical cells in culture. We also examined this question using isolated adrenocortical cells obtained from normal adult male rats. We found that a variety of antimicrotubular drugs will stimulate steroid synthesis in a dose-dependent fashion with the maximal response being equivalent to that obtained with either ACTH or cAMP. The mechanism of this response remains to be elucidated. These results have been published in Endocrinology, 103: 1281-1288, 1978. (3) A study is in press BBA, 1979, comparing bovine brain and adrenal cortex (neural versus non-neural) microtubules purified through three-four cycles of assembly/disassembly. The comparison included (a) colchicine binding activity, (b) tyrosylation using a specific tubulin-tyrosine ligase (which adds a tyrosine residue to the C-terminal glutamate or glutamine of the α chain) in conjunction with carboxy-peptidase A (which recovers the tyrosine), (c) examination of so-called microtubule associated proteins on SDS-PAGE, and (d) percent protein and amino acid analysis using the autoanalyzer.

C. Significance: The role of microtubules in a variety of cell functions (e.g., mitosis, motility, cell shape, etc.) has been well-delineated. The microtubule organelle is found in all cells, although its highest concentration appears to be in neural tissue. How this ubiquitous organelle which plays a role in multiple cell activities is involved in such a highly specialized function as steroidogenesis is simply not known. This remains an interesting question in cell biology.

D. Future Plans: Elucidation of the microtubular and microfilamentation systems of the adrenal cortex in regulation of steroidogenesis currently suffers from a total lack of careful morphological correlative studies. Our plans call for the establishment of stable, homogenous population of adrenocortical cells in tissue culture. With such a culture system, we plan to perform a variety of morphological studies utilizing transmission electromicroscopy, scanning electromicroscopy, and immunofluorescent or ferritin tagging techniques.

III. Steroid Secretory Proteins. These studies have not progressed very far as yet but they are based on the theoretical consideration that steroids are released from specific cellular proteins during the secretory process.

IV. Stimulation of steroidogenesis by a soluble adrenocortical factor(s).

What has progressed to a considerable extent has been the examination of a soluble stimulatory factor(s) detected in the adrenal cortex following the in vivo administration of ACTH to hypophysectomized rats. This heat-sensitive factor will cause a 3-4 fold increase in the rate of pregnenolone formation using partially purified mitochondria obtained from hypophysectomized untreated litter mates. The stimulation is dose-dependent, and cytosol prepared from non-adrenal tissue does not contain any stimulating activity. Characterization of this activity is currently being completed.

Project Description

I. Non-catalytic steroid-binding proteins.

A. Rationale: The rate-limiting step in steroid synthesis in considered to be the conversion of cholesterol to pregnenolone (i.e., the cholesterol lyase reaction). This reaction occurs at an intramitochondrial site (possibly associated with the outer membrane). Cholesterol, which is an absolute requirement for steroid synthesis, is a ubiquitous sterol, being found in membranes and stored esterified to fatty acids in cytoplasmic lipid droplets; it can also be made available through de novo synthesis and extraction from the circulation. The exact location and pool size of cholesterol utilized in steroid synthesis are not known. It is presumed, however, that the principal source of cholesterol is extramitochondrial. If this is true, then there must be a mechanism for transporting cholesterol into the mitochondrion where the cholesterol lyase is located. The molecular events involved in this transport are not known. Similarly, pregnenolone, the product of the rate-limiting step must move to specific extramitochondrial sites for further enzymatic alterations leading to the formation of cortisol, aldosterone, or one of the sex steroids. The molecular events involved in this transport are also not known. It is conceivable that specific proteins acting as transport vehicles are involved.

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3. 20α-hydroxycholesterol-binding protein. The studies here are still preliminary.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00161-04 RR

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Role of Sex Steroids in Regulation of FSH and LH Levels in Man

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI R.J. Sherins, M.D., Senior Investigator DES, ERRB, NICHD, NIH

Other D.L. Loriaux, M.D., Ph.D., Chief, Developmental Endocrinology Section, ERRB, NICHD, NIH

COOPERATING UNITS (if any)

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Developmental Endocrinology Section

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	2	0

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A series of studies in the rat have been utilized to ascertain the extent to which testosterone and estradiol can regulate the secretion of FSH and LH in the absence of the testes. The data demonstrate that 1) testosterone alone at physiologic doses can maintain both gonadotropins within the normal range, 2) that estradiol alone preferentially suppresses LH, but not FSH concentrations and 3) that a monotropic increase in plasma FSH is produced when there is reduced androgen production in association with increased estradiol production. These data provide an alternate explanation for the inhibin hypothesis which suggests that there is a specific inhibitor of FSH secretion arising from the seminiferous tubules.

PHS-6040
(Rev. 10-76)

Objectives:

A considerable body of data exists suggesting that androgens regulate pituitary LH secretion in men by negative feedback inhibition. Estradiol, however, also significantly suppresses LH secretion. Since plasma estradiol in men is derived primarily from testosterone aromatized in peripheral tissues, any study of the role of testosterone per se on gonadotropin secretion measures the effect of both testosterone and estradiol. Accordingly, a series of studies have been developed to compare the relative contributions of testosterone to estradiol alone on the regulation of FSH and LH concentrations in normal men and male rats.

Methods Employed:

Testosterone (T) and Estradiol (E) were administered to castrate adult male rats via silastic capsules. Plasma levels of T, E, and FSH and LH were measured by radioimmunoassay from samples obtained weekly during one month of study.

Major Findings:

When T alone was given at a dose which maintained mid normal plasma T levels and normal prostate size, plasma FSH and LH were both normal, indicating that testicular factors other than sex steroids are not necessary for maintaining FSH and LH levels within the normal range in the rat.

By contrast, when E alone was given at a dose which increased plasma E levels slightly above normal, plasma LH concentrations were suppressed, while FSH levels remained unexpectedly elevated into the castrate range. The data indicate that androgen (not estrogen) is critical in maintaining tonic suppression of FSH secretion.

When T and E were administered together in doses which produced subphysiologic levels of plasma T, half normal prostatic size, and slight increase in plasma E, a monotropic increase in plasma FSH occurred similar to that seen in male animals with severe germ cell depletion.

Significance:

These data add considerable insight into the mechanism of control of gonadotropin secretion in the male. The results indicate that testosterone production within the normal range can maintain both gonadotropins within the normal range in the absence of other testicular factors and, further, that a monotropic increase in plasma FSH levels can be induced when there is subnormal replacement of T but increased production of E. The data provide an alternate explanation for the inhibin hypothesis.

Proposed Course:

1) Further studies in the castrate rat will be pursued to establish whether T and E act at the level of the hypothalamus to alter GnRh concentrations.

2) Using in vitro cultures of rat anterior pituitary cells, the effects of T and E on synthesis of FSH and LH will be investigated.

3) Using castrate men, men with germ cell depletion and men who are functionally castrate, a systematic study of androgen production in relationship to estrogen production will be made to determine if the monotropic increase in FSH seen in men with germinal aplasia can be accounted for by a decrease in T production in association with increased E production.

Publications:

Sherins, R.J., Olweny, C.L.M. and Ziegler, J.L.: Gynecomastia and Gonadal Dysfunction in Adolescent Boys Treated with Combination Chemotherapy for Hodgkin's Disease. New Engl. J. Med. 299: 12-16, 1978.

Winters, S.J., Janick, J.J., Loriaux, D.L. and Sherins, R.J.: Studies on the Role of Sex Steroids in the Feedback Control of Gonadotropin Concentration in Men, II: Use of the Estrogen Antagonist Clomiphene Citrate. J. Clin. Endocrinol., 48: 222-227, 1979.

Marynick, S.P., Loriaux, D.L., Sherins, R.J., Pita, J.C. and Lipsett, M.B.: Evidence that Testosterone Acts as Both an Androgen and an Estrogen in Suppressing Pituitary Gonadotropin Secretion in Men. J. Clin. Endocrinol., in press.

Winters, S.J., Sherins, R.J., and Loriaux, D.L.: Studies on the Role of Sex Steroids in the Feedback Regulation of Gonadotropin Concentration in Men, III: Androgen Resistance in Primary Gonadal Failure. J. Clin. Endocrinol., 48: 553-558, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00162-04 RR

PERIOD COVERED

October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Studies of a Specific FSH Inhibitor from Seminiferous Tubules

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI R.J. Sherins, M.D., Senior Investigator DES, ERRB, NICHD, NIH

COOPERATING UNITS (if any)

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Developmental Endocrinology Section

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Preliminary studies of aqueous extracts of porcine follicular fluid demonstrate a dose dependent suppression of FSH and LH in an in-vitro bioassay utilizing LRF stimulated gonadotropin release from neonatal rat pituitary cell primary cultures. This gonadotropin suppression does not appear to be due to sex steroid contamination. These data are encouraging for the hypothesis that a non-steroid FSH inhibitor (inhibin) is produced by the seminiferous tubule.

PHS-6040
(Rev. 10-76)

Objectives:

Much indirect evidence has accumulated during the past five years, in both rodents and man, to suggest that there is a specific seminiferous tubular inhibitor of FSH secretion (inhibin), independent of the regulation exerted via sex steroids of Leydig cell origin. Preliminary reports have appeared describing direct inhibition of FSH secretion by aqueous extracts of testis, rete testis fluid, bovine and porcine follicular fluid and seminal fluid which are devoid of contaminating sex steroid. The purpose of this project is to develop methods to isolate and chemically characterize this putative FSH inhibitor.

Methods Employed:

Sephadex G^{75} fractions of porcine follicular fluid are prepared.

The gonadotropin suppressive activity of these preparations is tested in an *in vitro* bioassay of neonatal rat pituitary cells grown in primary tissue culture. Tissue preparations are assayed for their ability to suppress gonadotropin release stimulated by the *in vitro* administration of LRF.

Major Findings:

Primary culture of rat pituicytes has proved to be a reliable and precise *in vitro* bioassay of LRF activity which is not significantly altered by 10^{-8} - 10^{-10} M concentration of sex steroid. Porcine follicular fluid has been shown to contain small molecular weight substances which produce dose-dependent suppression of LRF stimulated FSH and LH release. This suppression of FSH and LH release could be explained by a direct action of these substances upon the pituicytes, or by enhanced degradation of LRF or gonadotropin. Studies to answer these questions are in progress.

Significance:

These data suggest that testicular/pituitary interaction may indeed involve molecules other than sex steroids as classically described. Development of new concepts of FSH regulation is not only important in reproductive biology, but also may provide insight into mechanisms of altering reproductive function critical to such fields as contraception and infertility.

Proposed Course:

1. In collaboration with Dr. Harry Chen, techniques are being developed to isolate and characterize the non-sex steroid inhibitor from rete testis fluid, bovine and porcine follicular fluid. These tissues have been chosen because they appear to contain the inhibitor in high concentrations, simplifying the isolation procedure.

2. In collaboration with Dr. Gary Hodgen, an in vivo assay of the "FSH inhibitor" is being developed in the castrate Rhesus monkey.

Publications:

Lee, I.P., Sherins, R.J. and Dixon, R.L.: Evidence for induction of germinal aplasia in male rats by environmental exposure to Boron. Toxicol. Appl. Pharmacol. 45:577-590, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 HD 00163-04 RR

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)
Clinical Studies of Male Reproductive Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	R.J. Sherins, M.D., Senior Investigator	DES, ERRB, NICHD, NIH
Other	T. Mann, Visiting Scientist	ERRB, NICHD
	R. Jones, Visiting Scientist	ERRB, NICHD
	C. Gagnon, Visiting Scientist	ERRB, NICHD
	J.L. Ziegler, Senior Investigator	NCI, NIH
	N. Javadpour, Senior Investigator	NCI, NIH

COOPERATING UNITS (if any)
C.W. Bardin, M.D., Director, Population Council, Rockefeler University, New York

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Developmental Endocrinology Section

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5	4	1.0

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A variety of studies on the clinical disorders of male reproduction are in progress. These include 1) studies of th hormonal regulation of spermatogenesis in hypogonadotropic men, 2) the effect of high venous ligation in fertile men with varicocele, 3) analysis of several biochemical parameters of sperm metabolism in men with necrospermia and 4) longitudinal evaluation of the adverse effects of cytotoxic drugs on testicular function in men receiving chemotherapy for malignant disorders.

PHS-6040
(Rev. 10-76)

Objectives:

The primary objective of the project is to provide a rational basis for the diagnosis and treatment of the disorders of male infertility.

Methods Employed:

The evaluation of patients with infertility and/or hypogonadism begins with a thorough examination in our outpatient clinic. Subsequent admission to our metabolic ward is used when more detailed study is required. About 100 new patients are studied annually. A variety of techniques are employed which include: measurement of hormones (FSH, LH, testosterone, estradiol, etc.) in biological fluids, semen analysis, testicular biopsy, chromosome analysis, electron microscopy of sperm and/or testis, and selected enzyme activity measurements in sperm.

Major Findings:

Studies of semen from infertile men with non-motile sperm (necrospermia) show 1) that such cells fail to metabolize fructose, the normal primary energy source in sperm, and 2) have increased specific activity of an endogenous lipid peroxidase, capable of producing peroxides of sperm phospholipid. These peroxides promptly and irreversibly immobilize spermatozoa.

Human sperm have recently been shown also to contain protein carboxyl methylase, an enzyme required for chemotaxis by mammalian monocytes and bacteria. This enzyme acts upon a specific protein methyl acceptor and inactivation of the enzyme leads to failure of characteristic unidirectional motile progression. A deficiency of protein carboxyl methylase activity has been identified in some men with immotile sperm.

Significance:

These observations suggest that there may be a biochemical marker for certain of the male infertility states in which sperm motility is altered.

Proposed Course:

Pursue studies of human sperm biochemistry and attempt to identify correlations between the parameters of fructolysis, caffeine activation of motility, the specific activity of protein carboxyl methylase and lipid peroxidase among men with necrospermia as well as infertile men with less severe disorders of sperm motility. We will include electron microscopic evaluation of the sperm in view of recent findings in other laboratories that the sperm of some necrospermic patients show ultrastructural abnormalities (i.e., absence of the dynein arms of axial filaments, known to contain ATPase).

Most would now agree that semen analysis is often an inadequate and insensitive tool in judging the adequacy of an ejaculate for impregnation. Accordingly, we are evaluating a spectrum of biochemical assays of human sperm function in an attempt to refine our diagnostic abilities in this regard.

Publications:

Jones, R. Mann, T. and Sherins, R.J.: Adverse Effects of Peroxidized Lipid on Human Spermatozoa. Proc. R. Soc., London, 201: 413-417, 1978.

Jones, R., Mann, R. and Sherins, R.J.: Peroxidative Breakdown of Phospholipids in Human Spermatozoa, Spermicidal Properties of Fatty Acid Peroxides, and Protective Action of Seminal Plasma. Fertil. Steril. 31: 531-537, 1979.

Gagnon, C., Sherins, R.J., Mann, R., Bardin, C.W., Amelar, R.D. and Dubin, L.: Deficiency of Protein Carboxyl-Methylase in Spermatozoa of Necrospermic Patients. In: Testis, Development, Structure and Function. A. Steinberger and E. Steinberger (Eds.), Raven Press, in press.

Mann, T., Jones, R. and Sherins, R.J.: Oxygen Damage, Lipid Peroxidation and Motility of Spermatozoa. In: Testis, Development, Structure and Function. A. Steinberger and E. Steinberger (Eds.), Raven Press, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00164-03 RR

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Strategy and Mechanisms of Fractionation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	Nga Y. Nguyen	Visiting Scientist	ERRB, NICHD
Other	A. Chrambach	Senior Investigator	ERRB, NICHD
	L. Hjelmeland	Staff Fellow	DPB, NICHD

COOPERATING UNITS (if any)

None

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A combined PAGE and IFPA isolation strategy was devised.

PHS-6040
(Rev. 10-76)

A fractionation strategy for protein isolation has been devised, using optimization of a) pH, ionic strength, solvent and other "happiness factors" by Steady-State Stacking; b) Ferguson plot analysis to determine whether PAGE or EF are applicable; c) optimization of gel concentration by program T-OPT in the PAGE case, followed by its analytical and preparative application; d) maximal pH gradient flattening in the gel EF case, followed by its analytical and preparative application.

Plan for '80:

Measurement of resolution in EF as a function of pH gradient flattening. Application of apparent diffusion coefficients to the computer simulation of resolution by preparative elution PAGE (see also report by B. Chen). Multicomponent analysis with component identification in terms of x,y-pairs and their statistical limits.

Publications:

Chrambach, A.: PAGE and IFPA in the Study of Native Macromolecules. J. Molecular and Cellular Biology, 1979, in press.

Chrambach, A., Nguyen, N. Y., and Hjelmeland, L. M.: New Electrophoretic Approaches Applicable to Cell Separation. In Peeters, H. (Ed.): Separation of Cells and Subcellular Elements. Oxford, Pergamon Press, 1979, in press.

Chrambach, A.: Analytical Polyacrylamide Gel Electrophoresis in Multiphasic Buffer Systems ("Disc Electrophoresis"). In Righetti, P. J., van Oss, C. J., and Vanderhoff, J. W. (Eds.): "Electrokinetic Separation Methods." Amsterdam, Elsevier/North Holland Biomedical Press, 1978, pp. 275-292.

Chrambach, A., and Nguyen, N. Y.: A Unified View of Electrophoretic Charge Fractionation. In Catsimpoolas, N. (Ed.): "Electrophoresis '78." Amsterdam-New York, N. Y., Elsevier/North Holland Publishing Company, 1978, pp. 3-18.

Hjelmeland, L. M., Nebert, D. W., and Chrambach, A.: Electrophoresis and Electrofocusing of Native Membrane Proteins. In Catsimpoolas, N. (Ed.): "Electrophoresis '78." Amsterdam-New York, N. Y., Elsevier/North Holland Publishing Company, 1978, pp. 29-56.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00165-04 RR

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Isolation and Characterization of Protein Hormones and Other Active Proteins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	A. Chrambach	Senior Investigator	ERRB, NICHD
Other	H. C. Chen	Senior Investigator	ERRB, NICHD
	M. Ben-David	Visiting Scientist	Hebrew University[1]
	S. Ben-Or	Visiting Scientist	Hebrew University[2]
	N. Y. Nguyen	Visiting Scientist	ERRB, NICHD
	M. I. New	Professor	Cornell University[3]
	R. E. Grindeland	Senior Investigator	NASA[4]
	U. Lang	Visiting Fellow	NIAMDD
	B. An der Lan	Visiting Fellow	DBP, FDA
	E. Leonard	Senior Investigator	NCI
	L. Harrison	Visiting Scientist	NIAMDD
	L. Hjelmeland	Staff Fellow	DPB, NICHD
	N. Nwokoro	Visiting Fellow	ERRB, NICHD

COOPERATING UNITS (if any)
[1]Pharmacology Dept., Hadassah Medical School, Hebrew Univ., Jerusalem, Israel; [2]Physiology Dept., Hadassah Medical School, Hebrew Univ., Jerusalem, Israel; [3]School of Medicine, Dept. of Pediatrics; [4]Moffett Field, California.

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1) A procedure for preparation of hPRL isohormones B and C by isoelectric focusing was worked out. 2) Glucocorticoid receptor from the 2-week chick embryonic retina was physically characterized by PAGE. 3) A procedure for isolation of hGH isohormones D and E by electrofocusing was developed. The isolation procedure using preparative isotachophoresis was modified by electrophoretic extraction and concentration of the isohormones, but failed to provide homogeneous hGH-E and -D. 4) Insulin receptor was physically characterized by PAGE in Triton X-100. Characterization in other detergents expected to yield greater disaggregation without inactivation is in progress. 5) Tetanus toxin was physically characterized by PAGE and IFPA. Heterogeneity progressing as a function of time after radioiodination was demonstrated by IFPA. 6) Rationales for solubilization and disaggregation of membrane proteins based on micellar properties for separation by PAGE and IFPA were developed. 7) hCG CR119 standard was characterized by IFPA with regard to the constituent isohomones and their properties.

PHS-6040
(Rev. 10-76)

1) Isolation of hPRL isohormones (in collaboration with M. Ben-David): Using recent techniques for pH gradient flattening in IFPA, a procedure for isolating hPRL-B and -C was developed. Application of this procedure to isolation awaits the elaboration of new hPRL starting material from amniotic fluid.

2) Glucocorticoid receptor in the 9- and 15-day chick embryonic retina (in collaboration with S. Ben-Or): The receptor in the functional state (15 day) has been characterized by PAGE. To gain insight into the molecular aspect of function, the 9-day non-functional receptor still needs to be characterized for comparison.

3) hGH isohormone E isolation (in collaboration with N. Y. Nguyen, M. I. New, R. E. Grindeland): Preparative isotachophoresis failed to provide homogeneous product or good yield. A suitable IFPA procedure was elaborated. Application is in progress.

4) Insulin receptor (in collaboration with U. Lang and L. Harrison): The receptor in 0.1% Triton was characterized in PAGE with maintenance of activity. Its MW is approximately 1 million. Present studies aim at producing a smaller active species by use of other detergents and detergent mixtures.

5) Iodinated Tetanus toxin (in collaboration with B. An der Lan): Iodinated Tetanus toxin was characterized by PAGE and IFPA at various times after iodination. In PAGE, 15% of non-specific label can be removed. Otherwise, the molecule appears homogeneous (MW 150,000) and indistinguishable between various iodination procedures. In IFPA, the progressive appearance of an iodination artifact was demonstrated.

6) Detergent PAGE and IFPA with activity maintenance (in collaboration with L. Hjelmeland): Rationales for electrophoretic separations in detergents with maintenance of activity were elaborated based on the micellar properties of the detergent and detergent mixtures.

7) hCG CR119 (in collaboration with N. Nwokoro and H. C. Chen): Using an optimized pH gradient, up to 14 components were demonstrated by IFPA. Each was kinetically characterized by pI'. All are equally immunoactive, and vary in sialic acid content. The in vitro bioactivity of the 6 major components is the same; the in vivo bioactivities differ. Major components after IFPA isolation are homogeneous. Upon re-run, pI' values decrease by approximately 0.5 pH unit.

8) Macrophage stimulating factor (in collaboration with E. Leonard): An attempt is being made to replace a previous "multistep-zero yield" preparative procedure by a 2-step one. Focusing of crude serum containing the factor (pI=7) is the first step, preparative PAGE the second.

Publications:

Newby, A. C., Rodbell, M., and Chrambach, A.: Adenylate Cyclase in Polyacrylamide Gel Electrophoresis: Solubilized but Active. Arch. Biochem. Biophys. 190: 109-117, 1978.

Newby, A. C., and Chrambach, A.: Disaggregation with Enhanced Net Charge of Enzymatically Active Adenylate Cyclase in Mixtures of Ionic and Nonionic Detergents. Biochem. J. 177: 623-630, 1978.

Bauer, A. C., Chrambach, A., and Levenbook, L.: Molecular Forms of Four Aldolases from Larval and Adult Stages of the Blowfly Phormia regina (Meigen). Insect Biochemistry 8: 443-447, 1978.

Ben-David, M., and Chrambach, A.: A Method for Isolation by Gel Electrofocusing of Isohormones B and C of Human Prolactin from Amniotic Fluid. J. Endocrinology, 1979, in press.

Lang, U., Kahn, C. R., and Chrambach, A.: Characterization of the Insulin Receptor and Insulin Degrading Activity from Human Lymphocytes by Quantitative Polyacrylamide Gel Electrophoresis. Endocrinology, 1979, in press.

Nguyen, N. Y., and Chrambach, A.: A 3-Step Method for Isolating a Few to Several Hundred Milligrams of Protein. J. Biochem. Biophys. Methods, 1979, in press.

Hjelmeland, L. M., Nebert, D. W., and Chrambach, A.: Electrofocusing of Integral Membrane Proteins in Mixtures of Zwitterionic and Nonionic Detergents. Anal. Biochem. 95: 201-208, 1979.

Salokangas, A., Fabbro, D., Eppenberger, U., and Chrambach, A.: cAMP Dependent Protein Kinases, Their Subunits and cAMP-Binding Protein from Four Sources: A Comparison of Physical Characteristics Determined by Polyacrylamide Gel Electrophoresis. Eur. J. Biochem., 1979, in press.

Salokangas, A., Eppenberger, U., and Chrambach, A.: Isolation of Active cAMP Dependent Protein Kinases from Calf Ovaries: Gel Electrophoresis vs. Gel Electrofocusing. Eur. J. Biochem., 1979, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00170-03 RR

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)
A Randomized Study for the Treatment of Chromophobe Adenomas of the Pituitary Gland with Irradiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	Charles A. Strott	Senior Investigator	NICHD, ERRB
Other	C. Ronald Kahn	Senior Investigator	NIAMDD, DB
	Jesse Roth	Chief	NIAMDD, DB

COOPERATING UNITS (if any)

None

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Since the natural history of chromophobe adenomas unassociated with visual impairment is uncertain, the approach to therapy in patients with this disorder is controversial. Complications often attributed to tumor growth may, in fact, be sequalae of the treatment administered, whether it be radiotherapy or surgery. The purpose of this project is two-fold: 1) to observe the natural history of untreated chromophobe adenomas in patients who do not present initially with visual disturbances and 2) to compare their clinical course with that of patients treated by one commonly employed modality, i.e., radiation therapy. Thus, patients will be randomized into two groups: one group will receive no therapy and one group will be treated with conventional pituitary irradiation. In a sense, each group is serving as a control for the other group. Over the study period specific clinical and laboratory abnormalities will be assessed in each group and compared in an attempt to help guide physicians in the treatment of this disorder.

PHS-6040
(Rev. 10-76)

Project Description:

Male and female patient less than age 65 who have carried the diagnosis of a pituitary tumor for less than one year and for which they have not received prior treatment are eligible. Patients with acromegaly and Cushing's Syndrome will not be studied. Minors will be admitted to the protocol only with their own and parental consent. If a defect in visual fields using a one millimeter white object is found the patient will receive treatment in a standard fashion and will not be considered for this protocol. In addition, patients with the following conditions will be excluded from the protocol: 1) severe, incapacitating headaches; 2) complete erosion of the inferior margin (floor) of the sella turcica; 3) clinical evidence of cavernous sinus involvement (e.g., painful opthalmoplegia).

Historically, conventional radiation therapy has played a major role in the management of chromophobe adenomas of the pituitary. Complications such as optic neuritis, extraocular muscle paralysis and loss of memory which might be attributable to therapy are rare. When further endocrine hypofunction follows pituitary radiation, the relative contributions of the radiation and the underlying adenoma itself cannot be separated. This question could be answered in this prospective study of treated and untreated patients.

The purpose of this project is then to observe the natural history of untreated chromophobe adenomas which have not initially produced a visual field defect and to compare their clinical course with that of patients receiving conventional pituitary irradiation. Through a comparison of the long-term clinical and laboratory follow-up of these patient populations, a meaningful analysis of the relative impact of pituitary irradiation on the natural history of this disorder when left untreated could be made. In addition, specific clinical and laboratory abnormalities which will guide the physician in his treatment of these patients may be identified.

Major Findings:

This project has not been in existence very long, and since it will eventually involve a large number of patients followed for at least ten years, no conclusions can be made as yet. To date, four (4) patients have been randomized.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00171-03 RR

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Other PAGE Instrumentation and Procedures

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	A. Chrambach	Senior Investigator	ERRB, NICHD
Other	J. Peterson	Chem. Engineer	BEIB
	W. Dehn	Glass Technologist	BEIB
	B. An der Lan	Visiting Fellow	DBP, FDA
	N. Y. Nguyen	Visiting Scientist	ERRB, NICHD
	J. DiFonzo	Mech. Engineer	Hoefer Scientific
	L. Hjelmeland	Staff Fellow	DPB, NICHD

COOPERATING UNITS (if any)

Hoefer Scientific Instruments Company, San Francisco, California
Cornell University Medical School, Department of Pediatrics

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The following apparatus and procedures were elaborated: 1) A redesigned cell for measurement of segmental voltages; 2) An improved design and construction of the apparatus for the simultaneous PAGE analysis in different buffers or detergents; 3) A 10-chamber apparatus for protein extraction from gels and concentration, using Steady-State Stacking; 4) A PAGE apparatus insert for tubes of rectangular cross-section; 5) An autoradiography device for gel tubes; 6) A procedure of gel slicing applicable to "non-restrictive" electrofocusing gels; 7) Study on the effect of covalent bonding of polyacrylamide to glass tubes on pH gradient instability in IFPA; 8) Electrofocusing of membrane proteins in zwitterionic detergent.

PHS-6040
(Rev. 10-76)

1) A redesigned cell for measurement of segmental voltages (in collaboration with J. Peterson and W. Dehn): The mechanical stability and insulation of electrode wires of the apparatus for measurement of segmental voltage in electrofocusing proved inadequate upon prolonged use. A new cell was designed consisting of Pt sheet and Pyrex tube segments which are fused.

2) An improved design and construction of the apparatus for the simultaneous PAGE analysis of gels at various pH or detergent composition (in collaboration with W. Dehn): An all-Pyrex construction of the apparatus described previously (Newby, et al., see below) with improved heat-transfer and stability characteristics was designed, built and tested. It has now replaced the previous model in routine laboratory application.

3) A ten-chamber apparatus for the simultaneous extraction from gels, and concentration, of proteins (in collaboration with J. DiFonzo and N. Y. Nguyen, with the technical assistance of C. Fakunding): This is a modification of the apparatus for electrophoretic protein concentration reported in 1978 (with H. Wachslicht). It allows for protein isolation at the milligram to 1 gram level by excision of as many as 10 protein zones from a gel, and their simultaneous electrophoretic extraction and concentration. Yields of 200 mg loads of BSA and ovalbumin, and 20 mg loads of hGH, were 70-80%; purity of product after gel filtration was 80-90%.

4) A PAGE apparatus insert for tubes with rectangular cross-section (in collaboration with B. An der Lan): An insert for rectangular cross-section tubes (2 x 4 mm) fitting the optimized, versatile apparatus described previously (1976) was designed for the purpose of direct autoradiography of gels contained in the tube (see 5).

5) Cassette for direct autoradiography of gel tubes (in collaboration with B. An der Lan): A light-proof autoradiography cassette for gel tubes of rectangular cross-section (see 4) was designed and constructed to avoid the need for gel slicing and isotope analysis of individual gel slices as well as longitudinal sectioning and drying of sections into filter paper.

6) Procedure for transverse slicing of "non-restrictive" polyacrylamide gels (in collaboration with B. An der Lan): A procedure was developed which allowed sectioning of low %T gels, using the Hoefer Instruments electric gel slicer, which is otherwise unsuitable for such gels. By placing the gel on a soft support, which the sectioning blade can cut, clean 1 mm slices can be obtained from 5 %T, 15 $\%C_{DATD}$ and from 3.5 %T, 2 $\%C_{Bis}$ gels. The soft support is molded from "Placticine," an oil based modelling clay. The ends of the gels are supported by plugs of this material to prevent them from being distorted by the weight of the blade.

7) Elimination of wall separation as being contributory to pH gradient instability in IFPA (in collaboration with B. An der Lan and N. Y. Nguyen): Polyacrylamide gel covalently bonded to glass walls by Silane A-174, a vinyl-Si-methoxy compound, exhibits on electrofocusing with either buffer or Ampholine carrier ampholytes the same instability as control gels. Thus, wall separation is not contributing to pH gradient decay as had been postulated previously.

8) Electrofocusing in presence of a zwitterionic detergent (in collaboration with L. Hjelmeland): Sulfobetaine-14, a zwitterionic detergent, allows for IFPA of water-insoluble hydrophobic proteins, does not influence the pH gradient in IFPA, condenses across the entire pH gradient as predicted, but fails to maintain activity of the representative membrane protein, cytochrome P-450.

Plans for '80:

The replacement of multiple 18 mm diameter tubes in preparative IFPA by a single, 15-18 mm thick gel slab with optimized heat transfer characteristics. Development of a slicing device for this slab, and of a mechanism to translate Rf's from a stained (shrunk) guidestrip to the rest of the unstained gel slab.

Publications:

Newby, A. C., Mathews, G., and Chrambach, A.: A Simplified Polyacrylamide Gel Electrophoresis Apparatus for Simultaneous Application of Multiple Buffer Sytems or Detergent Combinations. Anal. Biochem. 91: 473-480, 1978.

Righetti, P. G., and Chrambach, A.: Hindered Migration of Amninoacids Toward Their Isoelectric Positions in Gel Electrofocusing, and Facilitation of the Migration at High Ionic Strength. Anal. Biochem. 90: 633-643, 1978.

Hjelmeland, L. M., Nebert, D. W., and Chrambach, A.: Electrofocusing of Integral Membrane Proteins in Mixtures of Zwitterionic and Nonionic Detergents. Anal. Biochem. 95: 201-208, 1979.

Magnusson, R. P., and Jackiw, A.: Automated Scanning of 14C on Polyacrylamide Gel. J. Biochem. Biophys. Methods 1: 65-68, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00173-03 RR

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Gel Electrofocusing Methods

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	N. Y. Nguyen	Visiting Scientist	ERRB, NICHD
Other	A. Chrambach	Senior Investigator	ERRB, NICHD
	B. An der Lan	Visiting Fellow	DBP, FDA
	L. Hjelmeland	Staff Fellow	DPB, NICHD
	A. Jackiw	Guest Worker	Wayne University
	N. Nwokoro	Visiting Fellow	ERRB, NICHD

COOPERATING UNITS (if any)

Department of Biochemistry, Wayne State University, Detroit

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1) Quantitative extraction of protein from EF slices in concentrated form was accomplished by Steady-State Stacking. 2) Parallel shifts of pH gradients along the pH axis were achieved by choice of the pK of the anolyte. 3) Stabilization of pH gradients was found to increase with the pK of the anolyte. 4) Inadequate wall adherence of polyacrylamide to glass under conditions of EF was ruled out as one of the previously postulated causes for pH gradient instability. 5) Conductance gaps in EF were demonstrated by a gel tube with Pt wires fused into its wall at 1 cm intervals and connected to a sliding wire potentiometer. All carrier constituent species exhibit such gaps in the neutral range. Their dynamics during EF were determined. 6) TMAPS was found to bridge conductance gaps in EF, at least temporarily, and to shift a neutral gap to the cathodic terminus at the steady-state.

PHS-6040
(Rev. 10-76)

1) Preparative Gel EF (in collaboration with A. Chrambach): By use of Steady-State Stacking, extraction and simultaneous concentration of protein in EF gel slices can be achieved in high yield and purity. Load range: 20-200 mg per component. Using a newly devised apparatus, 10 proteins can be extracted from their zones in gel EF simultaneously. Short alkalinization of the slices prior to extraction appears necessary.

2) pH Gradient engineering (in collaboration with A. Chrambach and B. An der Lan): pH gradients can be modulated by constituent addition and subtraction as shown previously. In addition, the acidity of the anolyte controls the pH gradient (see 3). This has both practical and mechanistic consequences.

3) Stabilization of pH gradients by choice of anolyte (in collaboration with A. Chrambach and B. An der Lan): Increasing the pK of the anolyte stabilizes pH gradients in EF. Lowering the pK of the catholyte is relatively ineffective.

4) Inadequate wall adherence is not a cause of pH gradient instability in EF (in collaboration with A. Chrambach and B. An der Lan): Covalent bonding of polyacrylamide to glass walls by vinyl-Si-trimethoxide (Silane A-174) does not abolish pH gradient instability neither in buffer nor in Ampholine focusing.

5) Conductance gaps in gel EF (in collaboration with A. Jackiw and A. Chrambach): All carrier ampholyte pI-ranges exhibit neutral conductance gaps. Their height increases with focusing time or voltage. Effects on resolution are beneficial in the region of enhanced voltage, and they are deleterious whenever the approach toward the isoelectric endpoint is blocked by the gap, or when the Joule heat in the region of the gap proves denaturing.

6) TMAPS as a conductance gap bridging agent (in collaboration with L. Hjelmeland and A. Chrambach): Trimethylamino-propionyl-sulfonate (TMAPS) condenses across the pH gradient in EF, and shifts the neutral conductance gap toward the cathodic terminus. But it does not abolish it at the steady-state. TMAPS homologues with more charged groups are being tested in the expectation to increase conductance at the pI to a level which abolishes the gap.

Plan for '80:

Compare hCG patterns in ultrafiltered vs. untreated Ampholine EF gels. Characterize serum hCG by gel EF. Test oligo-TMAPS for conductance gap bridging. Test copolymerized vinyl-ampholytes for the effect of stationary charges on resolution in PAGE and gel EF. Apply the preparative gel EF procedure (section 1) to proteins of biological importance, in particular hGH-E.

Publications:

Chrambach, A., and Nguyen, N. Y.: Preparative Electrophoresis, Isotachophoresis and Electrofocusing on Polyacrylamide Gel. In Righetti, P. J., van Oss, C. J., and Vanderhoff, J. W. (Eds.): "Electrokinetic Separation Methods." Amsterdam, Elsevier/North Holland Biomedical Press, 1978, pp. 337-367.

Nguyen, N. Y., and Chrambach, A.: Amino Acid Spacing in Isotachophoresis on Polyacrylamide Gel: A Critical Evaluation. Anal. Biochem. 94: 202-210, 1979.

Nguyen, N. Y., and Chrambach, A.: A 3-Step Method for Isolating a Few to Several Hundred Milligrams of Protein. J. Biochem. Biophys. Methods, 1979, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00174-03 RR

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Hormonal Control of Ovarian Proteoglycan Synthesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	D. Rodbard	Senior Investigator	ERRB, NICHD
Other	M. Yanagishita	Visiting Fellow	ERRB, NICHD
	V. C. Hascall	Senior Investigator	LB, NIDR

COOPERATING UNITS (if any)

Laboratory of Biochemistry, NIDR

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1.25	1.25	

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The proteoglycans present in porcine and rat follicular fluid have been characterized with regard to their chemical and physical-chemical properties. The proteoglycans, approximately 2 million molecular weight, consist of 20% protein, 55% dermatan sulfate and 20% oligosaccharides. The protein core, approximately 400,000 molecular weight, has significantly different amino acid composition and susceptibility to various proteolytic enzymes from those of cartilage-type proteoglycans. The dermatan sulfate chains are approximately 56,000 in molecular weight, and iduronic acid accounts for 9% of total hexuronic acid. The oligosaccharides, approximately 350 chains per molecule, consist of penta and hexasaccharides, and are linked to the protein core by the O-glycosidic bonds. The chemical composition of these oligosaccharides has been determined. The oligosaccharides resemble those found in mucin-type glycoproteins. There is no apparent change in proteoglycan molecules with the size of follicles. Further, we have demonstrated that in vitro cultured granulosa cells produce several species of proteoglycans in addition to those found in porcine follicular fluid.

PHS-6040
(Rev. 10-76)

Objectives:

This study is intended to examine biochemical mechanisms of ovarian responsiveness to follicle stimulating hormone, luteinizing hormone, human chorionic gonadotropin, and pregnant mares serum gonadotropin (PMSG). The molecular structure of ovarian proteoglycans (a unique constituent of the follicular fluid) is to be studied, as well as the effects of hormonal manipulations on the biosynthesis and physical-chemical properties of these proteoglycans.

Methods Employed:

1. Protein isolation, involving cesium chloride density gradient sedimentation under dissociating conditions, gel filtration involving Sepharose and Sephadex, polyacrylamide gel electrophoresis (PAGE) and SDS-PAGE.

2. Chemical characterization of protein and carbohydrate moieties of proteoglycans, using enzymatic, colorimetric, and chromatographic methods.

Major Findings:

Monomer proteoglycan was isolated from porcine ovarian follicular by isopycnic CsCl centrifugation in the presence of 4 M guanidine HCl and protease inhibitors. The elution profile of the D1 preparation on Sepharose 2B was similar to that of monomer proteoglycan from bovine nasal cartilage, indicating a similar molecular size. Follicular fluid proteoglycans consist of about 20% protein, 55% dermatan sulfate and 20% oligosaccharides rich in sialic acid, galactose, mannose, glucosamine and galactosamine. The amino acid composition of this proteoglycan is significantly different from that of cartilage proteoglycans, with a higher proportion of aspartic acid, threonine and lysine and lower amounts of proline and glycine. Alkali released dermatan sulfate chains are larger on Sepharose 6B (average MW = 56,000) than chondroitin sulfate chains from cartilage proteoglycans (average MW = 25,000), and iduronic acid accounts for 9% of total hexuronic acid. Disaccharide units released by chondroitinase ABC consists of 67% 4-sulfated, 22% 6-sulfated, 5% non-sulfated and 5% disulfated disaccharides. After treatment with 0.05 M NaOH, 1 M $NaBH_4$ at 45° for 25 hr, two major sialic acid containing oligosaccharides were observed on Sephadex G-25, corresponding to penta- and hexasaccharides. The pentasaccharide contained sialic acid, galactose, glucosamine and galactosamine in the proportions 1:2:1:1. The galactosamine is O-glycosidically linked to the protein core. This oligosaccharide accounts for approximately 77% of all the sialic acid in the follicular fluid proteoglycans. The hexasaccharide fraction contained sialic acid, galactose, mannose and glucosamine in the proportions 1:2:1:2. It also contained a small amount of fucose and galactosamine. The linkage of these oligosaccharides to the protein core remains to be determined. The follicular fluid proteoglycans, unlike those from cartilage, do not interact with hyaluronic acid. Digestion with trypsin, chymotrypsin or plasmin released dermatan sulfate-peptides nearly as small as those released by papain or alkali; in contrast, cartilage

proteoglycans were resistant to plasmin and released peptides containing an average of more than 4 chondroitin sulfate chains after trypsin or chymotrypsin digestion.

Studies by others (Strickland and Beers) have shown that the ovarian follicular fluid contains a high level of plasminogen activator immediately prior to ovulation. While this enzyme may conceivably be responsible for partial degradation of the follicular wall, it also appears likely that the soluble proteoglycans would also be affected. However, the apparent osmotic gradient which can be generated by complete proteolysis of the protein core, with release of the osmotically active polysaccharide side chains, is limited because of the low concentration of the proteoglycan in the follicular fluid. However, such proteolysis would also be responsible for a decrease in viscosity and, if similar proteoglycans are responsible for the formation of the cumulus oophorus, then such proteolysis may conceivably be responsible, in part, for the release of the ovum.

In related studies, we have demonstrated that in granulosa cell cultures derived from hypophysectomized immature female rats treated with diethylstilbestrol, or in granulosa cell cultures derived from animals previously treated with pregnant mares serum gonadotropin (PMSG), the addition of FSH in vitro results in a significant increase in 35_S sulfate incorporation into macromolecules. Using the same experimental system, we have also demonstrated that granulosa cells produce several different species of proteoglycans in addition to those found in porcine follicular fluid.

Proposed Course:

Further studies will focus on the in vitro model systems, utilizing granulosa cells from the HIFR-DES model and from the PMSG model. The role of proteoglycans in follicular growth, development, atresia and ovulation will be studied. Further, chemical characterization of the oligosaccharides and of the nature of biosynthesis of these materials will be studied.

Publications:

Yanagishita, M., Rodbard, D., and Hascall, V. C.: Isolation and Characterization of Proteoglycans from Porcine Ovarian Follicular Fluid. Journal of Biological Chemistry. 254(3):911-920, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00175-02 RR

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Gel Electrophoresis with Continuous Optical Scanning

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	Betty Chen	Staff Fellow	ERRB, NICHD
Other	David Rodbard	Senior Investigator	ERRB, NICHD
	Andreas Chrambach	Senior Investigator	ERRB, NICHD

COOPERATING UNITS (if any)

Massachusetts Institute of Technology, Cambridge, Massachusetts

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

By applying continuous optical scanning to polyacrylamide gel electrophoresis (PAGE), we have been able to study molecular properties and resolution parameters associated with protein concentration profiles which are taken while a protein is subjected to PAGE. The apparent diffusion coefficients, derived from band dispersion (zone width) of a concentration profile, were estimated under a set of chosen conditions for a relatively homogeneous native protein. β-lactoglobulin B, and also for its reduced subunit, and the SDS-derivative of that subunit. Further, an application of the apparatus to the separation and the identification of a oligocomponent system, such as alloantigens from ragweed pollen extract are currently being undertaken in this laboratory.

PHS-6040
(Rev. 10-76)

Objectives

Exploitation of the information on molecular structure and on resolution which can be derived from band width in PAGE.

Methods Employed

Continuous optical scanning of PAGE at 280 nm has been employed to monitor protein zone distribution and its position along a gel throughout electrophoresis. Absorbance data are recorded on a magnetic tape and subsequently are transmitted to the NIH central computing facility. Computer programs, CEPHAS and SCINT (written by B. Cole), are used to calculate the band dispersion (standard deviation) of a concentration profile, and to perform the automatic peak detection and peak analysis.

Major Findings

1. Estimation of the apparent diffusion coefficient of β-lactoglobulin B (in collaboration with A. Chrambach, and D. Rodbard).

Continuous optical scanning in PAGE permitted a reliable estimation of apparent diffusion coefficient, D', derived from the rate of band dispersion. Under a set of experimental conditions, D' was linearly related to current density and protein load. Log D' was linearly related to the gel concentration. However, D' was non-linearly related to the ionic strength. Extrapolation of D' to an ideal case, e.g., zero gel concentration, zero load, zero current, and "infinite" ionic strength, yielded an estimated free-diffusion coefficient, D, of 3.1×10^{-7} cm^2/s which is compatible with previously reported values.

2. The effect of SDS on band dispersion in PAGE (in collaboration with A. Chrambach).

Continuous optical scanning with PAGE was used to measure band dispersion (σ^2) of the reduced β-lactoglobulin subunit and of the SDS-derivate of the subunit using PAGE in the presence and absence of SDS. D' values calculated from the initial linear region of the relationship between σ^2 and time were 1.71×10^{-6} cm^2/s for D' without SDS and 7.1×10^{-7} cm^2/s for D' with SDS. The ratio of the above D' values, 2.4 is about twice the value calculated of 1.3 on the basis of a spherical model with the assumption that approximately 1.4 g of SDS are bound to each gram of protein. This indicates a better resolving power in terms of numbers of theoretical plates of SDS-PAGE as compared to PAGE.

3. Measurement of the degree of completion of the polymerization reaction of crosslinked polyacrylamide by spectophotometry (in collaboration with A. Chrambach).

The apparatus for continuous optical scanning during PAGE was applied to measuring the % conversion of UV-absorbing monomers to non-absorbing

polymer, taking advantage of its high intensity light source. Polymerization was carried out in the apparatus, and the decrease in absorbance was monitored continuously. The height of the residual absorbance plateau after polymerization was taken as a measure of residual vinyl monomers.

4. Application of continuous optical scanning to PAGE fraction of alloantigens (in collaboration with C. Anderson, H. Baer, and A. Chrambach).

Alloantigens from short ragweed pollen extract were subjected to continuous optical scanning with PAGE at pH 3.0. Within two hours from the start of electrophoresis, the same number (four) of major components was identified as by the far more time and labor-consuming conventional procedure, using fixation and staining. The study indicates that automated analyses of protein composition provide a rapid, accurate and efficient method for processing large numbers of samples routinely.

Proposed Course

Continuous optical scanning in PAGE will be applied to multicomponent protein samples, with automated analysis of protein composition in terms of relative electrophoretic mobility (Rf).

An attempt will be made to computer simulate the resolution between β-lactoglobulin B and its reduced subunit on elution PAGE and to verify the prediction experimentally.

Publications

Chen, B., Chrambach, A., and Rodbard, D.: Continuous optical scanning in polyacrylamide gel electrophoresis: Estimation of the apparent diffusion coefficient of β-lactoglobulin B. Anal. Biochem. 97: 120-130, 1979.

Chen, B., and Chrambach, A.: Estimation of polymerization efficiency in the formation of polyacrylamide gel, using continuous optical scanning during polymerization. J. Biochem. Biophys. Methods 1: 105-116, 1979.

Chen, B., and Chrambach, A.: Present applicability and limitation of the gel electrophoresis apparatus with continuous optical scanning. In Catsimpoolas, N. (Ed.): Electrophoresis '78. Elsevier, North Holland Publ. Co., 1978, pp. 19-28.

Chen, B., Griffith, A., Catsimpoolas, N., Chrambach, A. and Rodbard, D.: Comparison between continuous and multiphastic zone electrophoresis. Anal. Biochem. 89:609-615, 1978.

Chen, B., Rodbard, D. and Chrambach, A.: Polyacrylamide gel electrophoresis in multiphasic buffer systems with continuous scanning. Anal. Biochem. 89:596-608, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00176-02 RR

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Clinical Evaluation of Adrenocortical Hyperfunction

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI Charles A. Strott Senior Investigator NICHD, ERRB

COOPERATING UNITS (if any)

None

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A regimen of standard tests for the diagnosis and differential diagnosis of the hyperfunctioning pituitary-adrenal axis has been designed to provide data for comparison of the various determinations and procedures now commonly employed. In addition the results of these tests will be compared to investigative procedures to be carried out under protocols which will be separately submitted. Patients will be treated by standard clinical methods, or by investigative methods also to be covered by future protocols.

PHS-6040
(Rev. 10-76)

Project Description

The clinical features of the hyperfunctioning pituitary-adrenal axis, standard diagnostic tests, and principles of treatment have been elucidated by several centers and serve as the basis for management of most patients with Cushing's syndrome, primary aldosteronism, and the excessive production of androgens or estrogens. However, numerous problems remain. Such disorders are not common, but the symptoms and signs which can be associated with them, e.g., fatigue, weakness, hypertension, obesity, osteoporosis, hirsutism, glucose intolerance, etc., are relatively common; thus, evaluation for adrenal overactivity is likely, in many instances, to be unrewarding. Numerous "screening tests" which have been suggested are either unreliable, or excessively expensive. Although the history and physical examination are frequently adequate when dealing with a well-developed syndrome, the variety and subtlety of the presentations of these disorders are well-recognized. Recent experience on our ward has demonstrated that the usual baseline determinations and suppression tests repeated on the same patient may be variable and difficult to interpret. Periodic hormonogenesis is a feature of many of these disorders; thus, long hospitalizations and numerous determinations are frequently necessary.

Careful attention to the details of the various testing procedures and minor alterations in the performance of these tests have also led to a better understanding of the physiology of the normal hypothalmic-pituitary-adrenal axis and the pathophysiology of steroid secretion in disease states in which the adrenal may be primarily or secondarily involved. In addition, advances in the field of neuroendocrinology have made it desirable to continue to observe the behavior of certain peptides whose secretion is closely associated with that of adrenocorticotrophic hormone.

The present protocol is designed to compare several diagnostic tests commonly used in the evaluation of possible adrenocortical hyperfunction and to provide clinical material for the development of improved tests and procedures. The same patients will become the subjects of further protocols designed to delineate the pathophysiology of the various disease states and to evaluate the various modes of therapy. Until those protocols are operational, patients will be treated by standard clinical guidelines.

Patient selection will be directed primarily toward Cushing's syndrome, but the design of the protocol is general enough to include hypersecretion of any adrenocortical hormone. The basis for admission is the clinical suspicion of hypersecretion; but, when possible, appropriate steroid measurements will have been performed prior to admission.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00179-01 RR

PERIOD COVERED
October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)
Inferior Petrosal Sinus Catheterization in Diagnosis and Treatment of Pituitary Tumors.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	Charles A. Strott	Senior Investigator	NICHD, ERRB
Other	John R. Doppman	Chief, Diagnostic Radiology Dept.	CC

COOPERATING UNITS (if any)

None

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Adrenal

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	2	0

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Inferior Petrosal sinus opacification by retrograde venography has been used at several centers as an alternate technique for opacification of orbital veins. In addition, the procedure delineates the anatomy of the cavernous and intrasellar venous sinuses and provides an opportunity for obtaining blood samples directly from the cavernous sinuses. We propose that it may offer definitive methods for the diagnosis of pituitary tumors and that sampling the effluent for hormones will provide evidence for the source of hypersecreted hormones when that source is in doubt. To establish whether this be the case, patients with disorders of the pituitary-adrenal axis who are undergoing continuous venous catheterization for other reasons will have sampling of the inferior petrosal sinus for hormone measurement, and, when indicated opacification of the cavernous sinus.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00180-01 RR

PERIOD COVERED

October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Biology of Hormone Binding Proteins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	Bruce C. Nisula	Sr. Investigator	DES	ERRB	NICHD
Other	James F. Dunn	Clinical Associate	DES	ERRB	NICHD
	D. Lynn Loriaux	Head	DES	ERRB	NICHD
	Gordon Cutler	Sr. Investigator	DES	ERRB	NICHD
	Richard Sherins	Sr. Investigator	DES	ERRB	NICHD

COOPERATING UNITS (if any)

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Developmental Endocrinology Section

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The overall objective of this project is to understand the role of serum hormone binding proteins in normal physiology and in the pathophysiology of various human disease states. Novel methods with improved precision and sensitivity for the measurement of the principal human sex hormone transport proteins, testosterone-estradiol binding globulin and albumin, have been developed. Future directions of the project include the development and characterization of an animal model in which the physiologic role of the steroid binding globulins can be investigated and a detailed assessment of the nature of sex steroid transport throughout human development with particular emphasis on puberty and its disorders.

PHS-6040
(Rev. 10-76)

Objectives:

The overall objective of this project is to understand the biologic role of the high affinity hormone binding proteins which circulate in humans and apply this knowledge to the diagnosis and management of the various disease states in which derangements involving hormones with specific transport globulins occur. The research effort of the current year has been directed most heavily toward developing and standardizing the methodology necessary to accomplish these goals.

Methods Employed:

The levels of various steroids are measured by radioimmunoassay with separation procedures including column or thin layer chromatography employed as appropriate. The binding proteins are studied by gel chromatography, dialysis, or ammonium sulphate precipitation. Also, a lectin which binds glycoproteins is used to separate albumin from the high affinity globulin binding proteins by column chromatography or batch methods. Radiolabeled ligands are used in conjunction with Scatchard analysis to characterize the binding of relevant steroids to the binding proteins. Computer programs are used to solve the complex interactions between multiple ligands under physiologic conditions between unbound and various protein bound fractions in human serum.

Healthy normal volunteers and patients with various disease processes affecting the gonadal-pituitary axis have been the subjects of the initial phase of the project.

Progress:

The major advance of the current period has been the development and standardization of a new, efficient, and reproducible method for the measurement of the testosterone binding globulin, the principal sex hormone transport proteins in human serum. The technique utilizes a novel approach--adsorption of the serum testosterone-estradiol binding globulin onto a solid phase matrix of Concanavalin A Sepharose. Through appropriate Scatchard analysis of ligand binding to the protein in the solid phase, we can determine the association constant and the binding capacity of the testosterone-estradiol binding globulin. The procedure also permits determination of an albumin binding parameter equivalent to the product of its affinity and capacity for binding testosterone. In conjunction with the serum total testosterone concentration, these parameters can be used to calculate the in vivo distribution of the circulating testosterone in to unbound, albumin bound, and tesosterone--estradiol binding globulin bound fractions. The method is simple, requiring no special procedures and equipment not in routine use in laboratories where radioimmunoassays are performed. A technician can easily run nearly a dozen samples in an afternoon.

Our initial clinical studies with this new solid phase methodology have focused on an assessment of the agreement of our parameters and results for testosterone transport in healthy men and women with the more directly by others using steady state gel filtration. values reported by others. Indeed, the unbound testosterone concentrations calculated with our approach agree quite closely with those measured

We have completed one of a series of studies aimed at understanding the pathophysiology of gynecomastia. Pubertal gynecomastia is a transient phenomenon of several months duration, whereas pubertal macromastia, which can be defined as the development of female appearing breasts in otherwise normal males at puberty, persists into adulthood. A detailed analysis of the gonadal-pituitary axis and sex steroid transport in a group of 9 young men with pubertal macromastia was accomplished. We specifically sought to find evidence of increased estrogenic activity, or decreased androgenic activity. However, if an abnormal hormonal environment were responsible for the development of this condition, it must have resolved prior to the time that we studied these patients since aside from the breast development, their physical examinations were completely normal, and there were no demonstrable abnormalities in plasma sex steroid level, sex steroid transport, unbound sex steroid level, prolactin, frequency of gonadotropin increases following LHRH administration, and baseline gonadotropin level. Thus, the application of highly detailed and precise dissection of the pituitary gonadal axis in this clinical situation has revealed that fully developed adult-female type breasts are compatible with a normally functioning male pituitary-gonadal axis.

Significance:

The improved method for assessing testosterone transport developed as a part of this research effort could potentially have a substantial impact on the diagnostic approach used by physicians in their management of patients, particularly men, with reproductive problems. Since the vast majority of the circulating sex steroid (testosterone, dihydrotestosterone, and estradiol) is actually bound to either albumin or testosterone-estradiol binding globulin, and therefore not available to the peripheral tissues, diagnostic inferences drawn upon the total level of these sex steroids in plasma can be erroneous if there are alterations in the concentration of the binding proteins. The technique permits a precise determination of the levels and function of these binding proteins, and therefore, allows a level of diagnostic accuracy in the evaluation of male gonadal function which has heretofore not been generally available.

An in-depth study of young men with persistent pubertal macromastia has yielded some new insights to the pathophysiology of gynecomastia. It is of interest that having developed fully adult female-type breasts is compatible with a normally functioning male pituitary-gonadal axis. Still unresolved is whether such patients have normal function at the time of initial development of the macromastia.

Proposed Course:

From the clinical investigation side, our focus will be on characterizing the levels of steroid binding proteins throughout human development. Special attention is being given to the pubertal process, for which we will define the changes in binding capacity and binding affinity of the testosterone-estradiol binding globulin, the changes in albumin binding, total serum sex steroid levels, and the consequent changes in the bound and unbound hormone concentrations. Pubertal boys with physiological gynecomastia will be compared to those without in an attempt to define any differences in the sex hormonal milieux of the groups. Adults with idiopathic and other types of gynecomastia will also be studied.

A considerable amount of attention will be given to characterizing testosterone transport in men with testicular disorders. There is a particular interest in those conditions in which hypogonadism is partial or in which there is a discrepancy between the clinical findings and the total testosterone level. Klinefelter's syndrome and the Sertoli cell only type syndromes will be studied to give more definitive insights to the regulation of the gonadotropins.

Computer simulation of the distribution of over 20 steroid ligands among the three principal binding proteins for these hormones, cortisol binding globulin, testosterone-estradiol binding globulin, and albumin, is currently in progress. Our data for the parameters of binding and the available data concerning the blood levels of the endogenous steroid ligands, together with appropriate computer programs permit simulation of the steroidal environment of a variety of human conditions including childhood, puberty, senility, phases of the menstrual cycle, hypogonadism, pregnancy, and so on.

It is planned to attempt to develop and exploit a laboratory model for investigating the role of sex steroid binding proteins in the regulation of hypophyseal function and in the modulation of sex hormone action. The high affinity binding proteins will be partially purified for administration to laboratory species which lack such transport proteins. The consequences in terms of gonadotropin secretion, hormone kinetics, and hormone action will be assessed.

A systematic study of the structure-function relationships of the high affinity binding proteins will provide information not available in the literature concerning the interaction of a raft of drugs and chemicals, in addition to the endogenous steroids, with cortisol binding globulin and testosterone-estradiol binding globulin.

Publications:

1. Marynick, S.P., B.C. Nisula, J.C. Pita, and D. Lynn Loriaux: Persistent Pubertal Macromastia. Journal of Clinical Endocrinology and Metabolism, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00181-01 RR

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Steroid Antagonists

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	D. Lynn Loriaux, M.D., Ph.D., Head	DES, ERRB, NICHD, NIH
Other	G. Cutler, Jr., M.D., Sr. Investigator	DES, ERRB, NICHD, NIH
	G. Chrousos, M.D., Clinical Associate	PNMB, NICHD

COOPERATING UNITS (if any)
Marvin Karten

LAB/BRANCH
Endocrinology Reproduction Research Branch

SECTION
Developmental Endocrinology Section

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Clinically useful antagonists exist for estrogens, androgens, and mineralocorticoids. No antagonists have been identified for the glucocorticoids or the progestins. The objective of this project is to develop antagonists for both of these classes of steroids.

PHS-6040
(Rev. 10-76)

Objectives:

Clinically useful antagonists have not been developed for glucocorticoids or progestins. Antagonists for both of these classes of steroids exist *in vitro*. The objectives of this project are to understand why the known *in vitro* antagonists fail to act *in vivo*, and using this knowledge, to produce effective *in vivo* antagonists.

Methods Employed:

Methods consist of glucocorticoid and progesterone radioreceptor assays, *in vivo* tests of glucocorticoid action (thymust involution, glycogen deposition, growth retardation) and progesterone action (endometrial maturation index).

Progress:

11-deoxycortisol (S) is a known glucocorticoid antagonist *in vitro*. It has not previously been shown to be an antagonist *in vivo*. We found that the reason for the apparent failure of S to act as a glucocorticoid antagonist *in vivo* is that it undergoes 11 hydroxylation by the adrenal gland, thus being converted to an agonist. This has been confirmed by showing that S retains full antagonist activity in adrenalectomized rats. Work has progressed toward developing effective *in vivo* antagonists by developing S analogues that resist 11-hydroxylation, such as $\Delta^{1,9(11)}$S. This compound acts as a glucocorticoid antagonist both *in vivo* and *in vitro*.

Significance:

The early results of these studies suggest that it will be possible to develop effective antiglucocorticoids. These compounds, if effective in man, would have great promise as a therapeutic tool and as an adjunct to studying adrenal physiology.

Publications:

1. Murakami, T., D. Brandon, L. Loriaux, and M.B. Lipsett: Glucocorticoid Receptor in Polymorphonuclear Leukocytes: A Simple Method for Leukocyte Glucocorticoid Receptor Characterization. *J. Ster. Biochem.*, 10:475, 1978.

2. Murakami, T., D. Brandon, D. Rodbard, D.L. Loriaux, and M.B. Lipsett: Glucocorticoid Receptor in Circulating Mononuclear Leukocytes. *Endocrinology*, 104: 505, 1979.

3. Cutler, G.B., K.M. Barnes, M.A. Sauer, and D.L. Loriaux: 11-deoxycortisol: A Glucocorticoid Antagonist *in vivo*. *Endocrinology*, 104: 1839, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00182-01 RR

PERIOD COVERED
Oct. 1, 1978 - Sept. 30, 1979

TITLE OF PROJECT (80 characters or less)

Structure, Function, and Physiology of Glycoprotein Hormones

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	Bruce Nisula	Sr. Investigator	DES	ERRB	NICHD
Other:	Robert Wehmann	Clinical Associate	DES	ERRB	NICHD
	George Taliadouros	Visiting Fellow	DES	ERRB	NICHD
	Vishnu Purohit	Visiting Fellow	DES	ERRB	NICHD

COOPERATING UNITS (if any)

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Developmental Endocrinology Section

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The broad objective of this project is to understand the biology of the glycoprotein hormones, thyrotropin, luteinizing hormone, follicle stimulating hormone, and chorionic gonadotropin. Development of an extremely sensitive radioimmunoassay for chorionic gonadotropin in urine has facilitated the management of patients with neoplasms which secrete this marker. In addition, with this assay evidence has been obtained indicating that normal subjects excrete an hCG-like substance in urine. Studies of the metabolic clearance rates of the hCG subunits have shown that they have similar apparent initial volumes of distribution, but that the alpha subunit has much shorter half-lives than beta in both the rapid and slow phases to account for its more rapid clearance rate. More direct evidence that hCG interacts with the thyrotopin membrane receptor has been obtained. Future emphasis of the project will be on renal excretion and other mechanisms of disposal of the glycoprotein hormones and on the nature and etiologies of structural and functional heterogeneity in these hormones.

PHS-6040
(Rev. 10-76)

Objectives:

The overall objective of this project is to understand the biology of the glycoprotein hormones and the role of glycoprotein hormone derangements in human diseases. The research effort in the current year has been directed toward: 1) The development and exploitation of radioimmunoassays to unique immunologic determinants on the glycoprotein hormones, 2) The determination of the interaction of hCG and hLH with the thyrotropin receptor, 3) The investigation of the metabolism and distribution of the subunits of hCG with measurement of the parameters that describe their disappearance and estimation of their metabolic clearance rates.

Progress:

A highly sensitive radioimmunoassay for the measurement of hCG in human urine has been developed. The value of former radioimmunoassays for urine hCG has been limited principally because of cross-reactivity with human LH. By use of antisera to determinants on the intact hCG beta subunit and its carboxy-terminal peptide in conjunction with extensive purification and concentration techniques, it has been possible to measure hCG production in human subjects at levels ten fold lower than with former methods. The improved methodology has been applied to monitoring a number of patients with hCG-secreting malignancies and has been found to extend our ability to utilize this marker in managing these patients. One unusual, but not unexpected finding was that the urine of normal subjects contains a material with many of the properties of hCG. This material could be hCG, itself, as it contains the carboxy-terminal immunologic determinant, carbohydrate, and the conformational immunologic determinant of the beta subunit.

The development of a radioimmunoassay to a partially degraded product of TSH has made it possible to examine the role of such molecules in the pathogenesis of the ophthalmopathy of Graves' disease. This troublesome clinical problem has been studied in animals by other workers who find that pituitary extracts, such as TSH, cause exopthalmos in some respects similar to that observed in these patients. Indeed, human pituitary extracts contained large concentrations of partially degraded TSH, as did partially purified TSH. However, there was none of this product detectable in the sera of patients with Graves' ophthalmopathy, despite the fact that such patients' sera contains an exophthalmogenic factor when tested in animal bioassays. Clearly, partially degraded TSH does not account for the exophthalmogenic activity in serum. A more likely area for investigation of the etiology of exophthalmos is immunologic. In this regard, we have found that the immuno-suppressive agent, cyclophosphamide, produced improvement in the ophthalmopathy of 3 patients.

The glycoprotein hormones are composed of 2 dissimilar subunits which are designated alpha and beta. Work by many others has indicated that these subunits may be secretory products of the pituitary, the placenta, and various neoplasms. The kinetics of disappearance of the hCG subunits was studied in detail. Following intravenous injection, the subunit concentration was found to decline according to a 2 component exponential curve

model. In both components, the half-life of the alpha was much shorter than that of the beta, while the apparent volumes of distribution were essentially the same for the subunits. The estimated metabolic clearance rate of alpha was 90.5 ml/min and that of beta was 35.2 ml/min. Both of these are considerably greater than the metabolic clearance rate reported for intact hCG (4 ml/min).

Another glycoprotein which has been of recent interest is the enzyme dopamine beta hydroxylase which occupies a critical position in the scheme of sympathetic function. Because of its location within catecholamine granules, it is normally discharged from sympathoadrenal cells along with the catecholamines in a process called exocytosis. Exocytosis has been thought to be the primary source of circulating enzyme. Therefore, it has been postulated that the serum level of this enzyme would be a useful monitor of sympathetic activity. To examine this postulate, sympathetic activation was induced by insulin hypoglycemia in normal subjects. Interestingly, the serum dopamine-beta-hydroxylase level did not change despite intense sympathetic activation, indicating that the amount of enzyme released during acute sympathoadrenal activation is minute in comparison to the size of the extracellular compartment.

Further progress has been made in delineating the mechanism of the hyperthyroid state which occurs in association with trophoblastic disease. Using a highly sensitive TSH radioligand-receptor assay, TSH-competing activity was found in urine extracts obtained from hyperthyroid women but not euthyroid women with trophoblastic disease. Highly purified hCG also exhibited TSH-competing activity, and the levels of hCG in the urine concentrates of our hyperthyroid women were consonant with the activity in purified hCG. Thus, there was no evidence of a factor other than hCG as the cause of TSH-competing activity. Also, we have examined in detail crude hCG obtained from the urine of pregnant women and the derived highly purified hCG preparation in both TSH radioligand-radioreceptor assay and mouse thyroid bioassay, specifically searching for any evidence of a molecule, other than hCG itself, with thyroid-stimulating activity. None was found. If hCG has intrinsic thyrotropic activity, as these data indicate, then thyrotropic activity would be expected to be a secondary biological activity of LH, since there are such strong structural and functional similarities between hCG and LH. Indeed, our results with LH indicate that it has intrinsic thyrotropic activity as well.

Significance:

Our knowledge of glycoprotein hormone biology has been expanded by these studies. Our improved methodology for the measurement of hCG has already had important clinical application in the monitoring of patients with tumors that secrete this marker. New evidence to support the concept that hCG or some hCG-like material apart from LH normally circulates in healthy persons has been obtained. This has important implications not only

from the genetic point of view, but also from the practical side for the current research on immunization methods for fertility control. As a result of our studies on exophthalmos, the postulate that TSH or a TSH metabolite plays a pathogenetic role in this disease becomes increasingly less tenable. Other approaches to this problem deserve attention. Our data on the distribution metabolism, and degradation of the hCG subunits in humans has established the kinetic parameters for these substances, and raised some important questions about the determinants of metabolic clearance rates for this group of glycopeptides. The modification implied for the existing schema is that the sialic acid and carbohydrate contents are not the only determinants of the clearance rates of the glycoprotein hormones and their subunits, and suggest that molecular weight or size may be important or even predominant for the endogenous authentic molecules. A large body of largely circumstantial evidence has implicated hCG as the thyrotropic factor of pathogenetic significance in trophoblastic hyperthyroidism. Our recent data have reinforced this concept and provided a clearcut mechanism involving the TSH receptor. The interaction appears to derive its biochemical basis through the structural homologies between TSH, hCG and LH.

Proposed Course:

Future studies will give increasing emphasis to the distribution, disposition, metabolism, and degradation of the glycopeptide hormones. The initial focus will be on hCG and its subunits. The circulating moieties in normal pregnancy will be compared and contrasted to those apparent in normal volunteers administered the authentic, purified materials. Renal excretion as a pathway of disposal and the renal clearance rates for authentic hCG and subunits will be given examination. Continued attention to more accurate and sensitive methodology for the measurement of glycopeptide hormones and their related metabolites is expected. Structure-function studies will be pursued with more detailed analysis of the biochemical mechanisms of thyroid cell stimulation by various interstial cell stimulating hormones.

Publications:

1. Taliadouros, G.T., R.E. Canfield, and B.C. Nisula: Thyroid-Stimulating Activity of hCG and LH. Journal of Clinical Endocrinology and Metabolism, 47:855, 1978.

2. Nisula, B.C., and P.N. Fredlund: Radioimmunoassay for Partially Degraded Thyrotropin. Endocrine Research Communications, 5:125, 1978.

3. Ayala, A.R., B.C. Nisula, H.C. Chen, G.D. Hodgen, and G.T. Ross: Highly Sensitive Radioimmunoassay for Chorionic Gonadotropin in Human Urine. Journal of Clinical Endocrinology and Metabolism, 47:767, 1978.

4. Nisula, B.C., and J.M. Stolk: Serum Dopamine-β-hydroxylase Activity Does Not Reflect Sympathetic Activation During Hypoglycemia. Journal of Clinical Endocrinology and Metabolism, 47:753, 1979.

5. Wehmann, R.E., and B.C. Nisula: Metabolic Clearance Rates of the Subunits of Human Chorionic Gonadotropin in Man. Journal of Clinical Endocrinology and Metabolism, 48:753, 1979.

6. Bigos, S.T., B.C. Nisula, G.H. Daniels, R.C. Eastman, H.H. Johnston, and P.O. Kohler: Cyclophosphamide in the Management of Advanced Graves Ophthalmopathy. Annals of Internal Medicine, 90:921, 1979.

7. Davies, T.F., G.S. Taliadouros, K.J. Catt, and B.C. Nisula: Assessment of Urinary TSH-Competing Activity in Choriocarcinoma and Thyroid Disease: Further Evidence for HCG Interacting at the Thyroid Cell Membrane. Journal of Clinical Endocrinology and Metabolism, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00183-01

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Catechol Estrogens: physiological effects

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	D.L. Loriaux, M.D., Ph.D.	DES, ERRB, NICHD
Other	G. Merriam, M.D., Clinical Associate	DES, ERRB, NICHD
	S. Kono, M.D., Visiting Scientist	DES, ERRB, NICHD

COOPERATING UNITS (if any)

LAB/BRANCH
Endocrinology Reproduction Research Branch

SECTION
Developmental Endocrinology Section

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The catechol estrogens, 2 or 4 hydroxylated estrone and estradiol, represent major metabolites of the naturally occurring estrogens. Little is known of the origin, disposition, and metabolic effects of these compounds. The objective of this project is to address these questions.

PHS-6040
(Rev. 10-76)

Objectives:

Catechol estrogens have been identified in urine, and have been shown to be metabolic products of both estrone and estradiol. The biological effects of these compounds remains a controversial issue. The objectives of this project are to define the metabolic origin of the catechol estrogens, and to delineate their biological effects in man, using gonadotropin suppression as a response parameter, and in the rat, using uterine weight as an end point.

Progress:

The past one year has been devoted to the synthesis and purification of the reagents necessary to study this problem, including ^{14}C and ^{3}H - 2 and 4 hydroxy estradiol and estrone. In addition, large quantities of the non-radioactive catechol estrogens have been prepared for infusion studies in man.

Projected Course:

1. Each of the catechol estrogens will be infused into normal men in increasing doses to define the dose response curve of gonadotropic suppression for each compound.

2. Double isotope infusions of E_1 and E_2 with each of the catechol estrogens will be performed in normal men to define the origins of the catechol estrogens.

3. Agonist and antagonist activities of the catechol estrogens will be examined in the rat using uterine weight as a response parameter.

Publications:

none

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00184-01 RR

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Regulation of Pituitary Hormone Secretion

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	K. J. Catt, Chief	ERRB, NICHD
Other	R. L. Clayton, Guest Worker (MRC Fellow)	ERRB, NICHD
	Z. Naor, Visiting Fellow	ERRB, NICHD

COOPERATING UNITS (if any)

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Section on Hormonal Regulation

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project examines the mechanisms by which gonadotropin releasing hormone (GnRH) controls the secretion of LH and FSH by anterior pituitary cells. This includes characterization and analysis of the GnRH receptors in anterior pituitary tissue, and the role of cyclic nucleotides and other intracellular regulators in the mechanism of action of GnRH.

PHS-6040
(Rev. 10-76)

Objectives:

To characterize the GnRH receptors and elucidate the mechanism by which receptor occupancy leads to release of gonadotropic hormones.

Methods Employed:

Radioligand assay of GnRH receptors in pituitary homogenates and cultured cells. Measurement of gonadotropins and cyclic nucleotides by radioimmunoassay.

Major Findings:

1. Role of cyclic AMP in GnRH action.

The secretory response of pituitary gonadotropes to stimulation by gonadotropin-releasing hormone (GnRH) has been extensively studied, but the mechanism by which GnRH evokes gonadotropin synthesis and release has not been clarified. In particular, there has been conflicting evidence about the role of cAMP in GnRH-induced release of LH. To examine this question in more detail, the actions of GnRH on LH release and cAMP production were analyzed in primary cultures of collagenase-dispersed rat pituitary cells. In this system, addition of 10^{-10}-10^{-6}M GnRH to cultured pituicytes caused rapid release of LH into the incubation medium. In contrast, GnRH caused no significant change in intracellular or extracellular cAMP or in occupancy by cAMP of the regulatory subunit of protein kinase. Neither dibutyryl cAMP nor methyl isobutylxanthine (MIX) stimulated LH production to the same level as GnRH, and neither agent potentiated the effect of the releasing hormone. Cholera toxin and prostaglandin E_1 (PGE_1), both of which stimulated cAMP production in cultured pituitary cells, did not raise LH levels as markedly as GnRH. These results demonstrate the independence of LH release from cAMP accumulation in cultured gonadotropes, suggesting that cAMP is not required for stimulation of LH release from these cells and that GnRH acts on LH secretion by a different mechanism.

2. GnRH receptors and action.

A recently developed radioligand-receptor assay (RRA) for pituitary gonadotropin releasing hormone (GnRH) receptors, employing an iodinated superagonist analog as the radioligand, has been utilized to analyze the receptor binding properties of several GnRH analogs. This receptor assay is specific for GnRH and related peptides with agonist or antagonist activity. All superagonist analogs had equilibrium association constants (Ka) between 4 and 8-fold greater than that of the natural decapeptide (Ka = 6.6 x $10^8 M^{-1}$). Conversely, weak agonists and inactive analogs bound with affinities 20-300 times less than GnRH. Weak antagonists, such as [D-Phe^2]GnRH and [D-Phe^2,D-Phe^6]GnRH, had lower binding constants, while the most potent antagonist, [D-$pGlu^1$,D-Phe^2,D-$Trp^{3,6}$] GnRH, exhibited 8-fold higher affinity than GnRH for pituitary receptors. There was a strong positive correlation between receptor binding affinity and biological activity, whether agonist or antagonist, for the several GnRH analogs tested. The data from direct receptor binding analysis indicate that the three N-terminal amino acids are critical for receptor recognition, while the C-terminal des-Gly^{10}

ethylamide modification does not enhance binding affinity. This RRA provides a rapid and reliable method for screening of new analogs of GnRH for biological activity, and permits direct determination of the structural requirements for receptor recognition.

Significance:

Clarification of the mechanisms of action of GnRH should improve understanding of pituitary disorders and permit the development of improved methods for the control of gonadotropin secretion.

Proposed Course:

The role of cyclic GMP and calcium in GnRH action will be more extensively studied, and binding of GnRH to receptors in cultured pituitary cells will be compared with the resultant release of gonadotropins. The regulation and turnover of GnRH receptors will be analyzed during physiological changes in gonadotropin secretion *in vivo*, and during experimental changes *in vitro* in cultured gonadotropes.

Publications:

Conn, P.M., Morrell, D.V., Dufau, M.L., and Catt, K.J.: Gonadotropin-releasing hormone action in cultured pituicytes: independence of luteinizing hormone release and adenosine 3',5'-monophosphate production. Endocrinology 104: 448-453, 1979.

Clayton, R.N., and Catt, K.J.: Receptor binding affinity of GnRH analogs: analysis by radioligand-receptor assay. Endocrinology, 1979, in press.

NICHD Annual Report
October 1, 1978 through September 30, 1979
Child and Family Research Branch

Summary of Research Activities

During the past year a considerable amount of data has been collected in studies for which methods were being developed in the previous year. These investigations continued the Branch's exploration of early development and early environmental influences on the developing organism. Among the topics with which these studies were concerned were: the psychological aspects of childbirth; the early development of mastery motivation in normal and Downs Syndrome infants; family interaction during infancy, with special attention to the role of the father in families with an infant delivered by cesarean section, and in families in which the mother works; the influence on later adaptation of the infant's early attachment to the mother; and a cross-cultural study of caregiver-infant interactions. Several of these investigations use a common sample which enables study of issues which could not be investigated in a single study. For example, use of the childbirth sample in the mastery study will allow us to look at relations between childbirth experiences and mother-infant interaction at 6 and at 12 months. It will also enable us to study the role of early infant characteristics in the development of mastery behavior.

Most of our conclusions about the significance of childbirth events have been obtained through interviews after delivery. We have almost no data based on direct observation of the events surrounding childbirth, of the psychological supports given during labor and delivery. Moreover, in recent years fathers have become more directly involved in the births of their children. A study just completed has obtained data on the psychological aspects of labor by direct observation of the woman, her husband and the hospital personnel. In addition to obtaining substantive data on the labor, methodological data were obtained on the relation between the retrospective report of husband and wife on their experiences during labor and observations of labor by an impartial observer. Data have been collected on forty couples and their newborn infants. Interviews were conducted before delivery with the mother and father about their expectations of childbirth and after delivery about their perceptions and feelings about childbirth. Data were also obtained about their parenting behavior and their perceptions of the neonate's temperament through a scale developed in this laboratory, the Newborn Behavior Inventory. Infants were assessed by an independent observer using the Neonatal Behavioral Assessment Scale. Analyses are now in progress. The findings of this study should throw light on the early antecedents of parenting behavior, on how the experiences of labor and delivery influence parent-infant relationships during the first months of life. In addition, it will add to our knowledge of the interactions between psychological experiences and medical care during labor and neonatal status. The methods developed in this study are being used in a companion study which is about to be started. This study will compare infants born at home with infants born in the hospital.

Recognizing that cognitive development is only one small aspect of the infant's growth, we have begun to investigate the development of mastery motivation in infancy. In the study of mastery motivation, data collection on 69 infants at 6 and 12 months has been almost completed. Procedures for measuring mastery in 6 and 12 month infants were developed. Measures of parent-infant interaction have also been designed to study in fine detail mutually reciprocal interactions, from mother to infant and infant to mother. Interobserver reliability for coding mastery behaviors has been obtained; satisfactory reliability in the coding of home observations of the motherinfant and father-infant interaction has also been achieved. Data reduction is underway in preparation for analysis. Using the same procedures, Downs Syndrome infants are also being studied. The latter study is designed to ascertain the the ways in which Downs Syndrome infants differ from normal infants in their motivation to master the environment. The studies of mastery should throw light on the beginnings of the infant's active attempts to learn about the environment, to obtain feedback from it, to persist in practicing emerging skills and in problem-solving. Investigation of the relations between the early environment and the development of mastery motivation should have significance for parent education and for intervention programs. Among the theoretical and practical implications are its potential for clarifying the ways in which specific aspects of the early environment influence the development of mastery behaviors and competence during infancy.

Although it has long been recognized that the young child's relationship with the mother may serve as a prototype for relationships with other people, we still have little predictive data. A followup study of children at 3 years who had been first seen at 12 months revealed that preference for the mother at one year was significantly related to social and cognitive functioning at 3 years. In an attempt to investigate further some of the factors influencing socioemotional development, plans are underway to study the social adaptations of the children in the childbirth and mastery samples at 3-4 years of age.

Our work in parent-infant interaction has been confined to several social class groups within the American culture. Recently an opportunity arose to extend our knowledge of caregiver-infant interaction across cultures. Films from Micronesia made in connection with research of the National Institute of Neurological Diseases and Blindness became available to us. Procedures have been developed for coding these films of infant-caregiver interaction. Comparisons are being made of films of American infants and their caregivers. Coding of these films has been completed. Demonstration of the feasibility of these coding procedures is important in that it opens possibilities for using ethnographic film libraries for studying cultures which have undergone marked transitions in recent years.

The studies of family interaction that have been initiated this year are examining several questions that emphasize the close tie between the theoretical and the applied. We have extended our studies of the father's role in infancy to examine his role under the special circumstances of cesarean delivery and maternal employment. Procedures for observation of family interaction have been developed and inter-

observer reliability has been obtained. Collection of data has begun and will continue during the next fiscal year. To provide a cross-cultural comparison, a sample comparable in socioeconomic status is being studied in Denmark. Families are seen when the baby is 3 months old and again when the infant is 12 months of age. At both ages the parents are interviewed and asked to complete an instrument developed in this laboratory, Perception of Baby Temperament Scale, a revision of the Carey Temperament Scale. The psychometric properties of the Perception of Baby Temperament Scale had been investigated in a previous study and revised for the present investigation. The study of children who have had cesarean births will focus on whether there are differences in patterns of family interaction between these families and families with normal births, and if differences persist through the first year. The study on maternal employment will add to our understanding of how maternal employment influences the interactions of both parents with an infant and whether the mother's employment has significant immediate and longterm impacts on the father's relationship with the infant. These are issues on which there is increasing concern currently in American families and on which we have virtually no information in families with young infants.

Professional Activities of Staff:

Members of the Child and Family Research Branch have been guest lecturers at universities in the U. S and abroad. They have served as consultants on research to universities and to foundations. They have served on committees of professional organizations and on the editorial boards of a number of journals and they have reviewed manuscripts for publishers.

Nancy Gist was Guest Lecturer at the Institute for Child Study, Graduate School of Education, University of Maryland.

Dr. Robert Klein was an ad hoc reviewer for the journal, Developmental Psychology, and contributed a review of the book, "Malnutrition, Behavior and Social Organization," to Child Development Abstracts and Bibliography.

Joanne Nicholson is a member of the Committee on Health of the Division of Health Psychology, the American Psychological Association. She has been serving as coeditor of the Newsletter for Psychologists in Family Medicine and Primary Care.

Dr. Frank Pedersen initiated and carried out a cross-cultural study of family interaction at the University of Aarhus in Denmark. While engaged in this study he gave a series of lectures at Aarhus University. He completed the editing of a book, "The father-infant relationship: Observational studies in the family setting" which is to be published by Praeger, a division of Holt, Rinehart & Winston.

Dr. Kay Standley continues to serve on the Board of Convention Affairs and the committee on Continuing Education of the American Psychological Association.

Dr. Peter Vietze was a consultant to the Department of Pediatrics at the University of Cincinnati Medical School, to the Pediatrics Department, College of Medicine, Vanderbilt University and to the John F. Kennedy Center for Mental Retardation, Peabody College. He has served on the dissertation committees of students at Peabody College. He has reviewed grant proposals for the National Science Foundation and for the Canada Council, and has been an ad hoc reviewer for the journal Child Development.

Dr. Martha Zaslow was an ad hoc reviewer for the journal Developmental Psychology.

Dr. Leon Yarrow continues to serve on the Board of Directors of the Easter Seal Research Foundation and the National Center for Clinical Infant Programs. He also serves on the editorial boards of the Merrill-Palmer Quarterly Journal of Behavior and Development, and the Monographs of the Society for Research in Child Development. He has been an ad hoc reviewer for Developmental Psychology, Child Development, Human Development, Infant Behavior and Development, and the Journal of Child Psychology and Psychiatry. He has been a consultant on research grant applications to the Canada Council, the National Science Foundation, the W. T. Grant Foundation and the March of Dimes.

Publications Not Directly Related to any Specific Projects.

Altemeier, W. A., Vietze, P. M., Sherrod, K. B., Sandler, H. M., Falsey, S., and O'Connor, S. Prediction of child maltreatment during pregnancy. Journal of Child Psychiatry, 1979, 18, 205-218.

Marcus, B. B., Ruttle, K., and Vietze, P. M. Relationships among attachment behaviors during reunion at 13 and 32 months of age. Psychological Reports, 1979, 45, 59-62.

McLaughlin, J., Sandler, H. M., Sherrod, K., Vietze, P., and O'Connor, S. M. Social-psychological characteristics of adolescent mothers and behavior characteristics of their first-born infants. Population, 1979, 2, 69-73.

O'Connor, S., Altemeier, W. A., Sherrod, K. B., Sandler, H. M., and Vietze, P. M. The effect of extended postpartum contact on problems with parenting: A controlled study of 301 families. Birth and the Family Journal, 1978, 5, 231-234.

O'Connor, S., Vietze, P., Sherrod, K., Sandler, H., and Altemeier, W. How does rooming-in enhance the mother-infant bond? Pediatric Research, 1979, 13, 336.

Switzky, L. T., Vietze, P., and Switzky, H. Attitudinal and demographic predictors of breast-feeding and bottle-feeding behavior in mothers of six-week-old infants. Psychological Reports, 1979, 45, 3-14.

Yarrow, L. J. Historical perspectives and future directions in infant research. In J. Osofosky (Ed.), The Handbook of Infant Development. New York: Wiley, 1979, pp. 897-917.

Yarrow, L. J. Counsel for counselors. Contemporary Psychology, 1979, 24, 399-401.

Yarrow, L. J., and Anderson, B. Problems in procedures for exploring parent-infant interaction. In S. Trotter and E. Thoman (Eds.), Social Responsiveness of Infants. Brunswick, New Jersey: Johnson and Johnson, 1978, pp. 38-43.

Yarrow, L. J., and Anderson. B. Procedures for studying parent-infant interaction: A critique. In E. J. Thoman and V. Denenberg (Eds.), The Origins of Infant Social Responsiveness. Hillside, N.J.: Erlbaum, 1979, pp. 209-223.

Presentations Not Directly Related to Specific Projects.

Vietze, P. Assessing the developmental outcome of high-risk babies. Workshop on Maternal and Child Health in Tennessee: Common Concerns and Practical Approaches, Crossville, Tennessee, October, 1978.

Vietze, P. Follow-up of high risk babies: Identifying risk, establishing priorities and approaches for providing family support and educational intervention. With E. Badger and M. Foster Workshop on Maternal and Child Health in Tennessee, Crossville, Tennessee, October, 1978.

Vietze, P. M., O'Connor, S. M., Falsey, S., and Altemeier, W. A. Effects of rooming-in and parity on maternal attachment behavior. Presented at the meetings of the American Psychological Association, Toronto, Ontario, August, 1978.

Vietze, P. M., Sandler, H. M., and Falsey, S. Transactional approach to prediction of child maltreatment. Presented at the Biennial Meeting of the Society for Research in Child Development, San Francisco, Ca., March, 1979.

Vietze, P. M., Altemeier, W. A., Falsey, S., Sandler, H. M., and O'Connor, S. Prospective study of mothers at risk for child maltreatment. Presented at the International workshop on the "At Risk" infant, Tel Aviv, Israel, July, 1979.

Yarrow, L. J. The problem of environmental continuity. Presented to the Psychobiology Research Seminar, University of Colorado School of Medicine, April, 1979.

Yarrow, L. J. Reactions to separation from a mother-figure. Presented at Grand Rounds, Department of Psychiatry, University of Colorado School of Medicine, April, 1979.

Yarrow, L. J. Environmental discontinuity associated with transition from foster to adoptive home. Presented on symposium on Environmental Continuity and Discontinuity at the Biennial Meetings of the International Society for the Study of Behavioral Development, Lund, Sweden, June,, 1979.

Zaslow, M., Sostek, A. M., Vietze, P., Kreiss, L., van der Waals, F., and Rubenstein, D. Cross-cultural differences in expressiveness in caregiver-infant interaction: An analysis of filmed behavior on the Micronesian Island of Fais and in the United States. Presented at Society for Cross-Cultural Research, Charlottesville, Va., February, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00012-03 CFR

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Development of Mastery Motivation in Infants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. M. Vietze	Senior Staff Fellow	CFR, NICHD
	L. J. Yarrow	Chief, Child and Family Research	CFR, NICHD
OTHER:	R. M. MacTurk	Research Psychologist	CFR, NICHD
	M. E. McCarthy	Research Psychologist	CFR, NICHD
	S. McQuiston	Research Psychologist	CFR, NICHD
	D. Lindemer	Research Psychologist	CFR, NICHD

COOPERATING UNITS (if any)

None

LAB/BRANCH

Child and Family Research Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	4.75	0.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The major objective of the project is to investigate the development of mastery motivation in the first year of life. In a previous study, (Z01 HD 00008-04) three aspects of mastery were distinguished: persistence in problem solving, in practicing emerging sensorimotor skills, and in obtaining sensory feedback from the physical environment. The interrelationships among these three components of mastery motivation are being explored. The origins of mastery in parent-child transactions and its continuity over time are being studied. The sample includes 69 infants from middle income families. Several methods are being employed--a series of twelve tasks devised to measure mastery motivation; mother-infant observation and father-infant interaction at home; Bayley Scales of Infant Development; and several self-report measures of parent-infant interaction. Analyses will focus on continuity in mastery behavior between 6 and 12 months and on the relationship between the development of mastery and parent-infant interaction.

Project Description:

Objectives: The major goal of this project is to investigate the ontogeny of three facets of mastery motivation during the first year of life: persistence in problem solving, in practicing emerging sensorimotor skills, and in obtaining feedback from the physical environment. This research builds on a previous investigation in which measures of mastery motivation were developed for 12-month-old infants (Z01 HD 00008-04). In the present study we have revised and extended these measures, and have developed measures to study mastery at a younger age--6 months. A second purpose of the project is to examine the environmental correlates of mastery motivation. We have been observing mother-infant and father-infant interaction when the infants are 6 and 12 months of age to ascertain the significance of the infant's early experiences with his/her parents and with objects for the development of mastery motivation.

Methods Employed: The subjects for this study include 69 first-born infants from intact middle-income families (37 boys and 32 girls); only infants born after uneventful pregnancies and delivieries were included. Infants and parents were studied when the infants were 6 months of age and again when they were 12 months old. Data were obtained in several sessions at each age. During the first session, the infants were given the Bayley Scales of Infant Development. During the second laboratory visit, six of the mastery motivation tasks were administered; the other six tasks were given on the third visit. To allow for estimates of the internal consistency of the procedure, the order of presentation of the two sets of tasks was counter-balanced. Three observations of parent-infant interaction in the home were made within a week of the laboratory sessions. In addition, the parents were asked to keep a diary of the infant's activities for a week and to complete the Perception of Baby Temperament Scales. At 15 and 16 months of age, the mothers and infants are being observed in a separation procedure adapted from a previous study (Z01 HD 00003). This will allow us to examine the relationships between mastery motivation and mother-infant attachment as well as to study the influence of early mother-infant interaction on the infant's later attachment to the mother.

The three components of mastery motivation were assessed in a variety of ways. The Practicing Sensorimotor Skills component consists of tasks in which it is necessary to coordinate perceptual and fine motor skills, an activity in which infants often engage repetitiously. An example of one of the items for 6 month olds is a toy in which three plastic "men" in a small tub are presented, the infant's task being to remove the men from the tub and then replace them. A similar task for 12 month olds is a peg boat from which the infant can remove pegs and replace them. The Problem Solving tasks include a variety of situations in which an object is located out of direct reach of the infant, but within view. The infant is encouraged to obtain the object. For the 6 month olds, one of the problem solving tasks consists in presenting an attractive toy just beyond the infant's reach. The toy is on a piece of cloth and can be obtained only by pulling the cloth towards him. A 12-month problem solving task involves showing the child a transparent door behind which is a toy that can be

obtained only by correct manipulation of the latch. Effectance Production tasks are those in which the infant produces an effect by his own action. At 6 months, one of the Effectance Production tasks consists of an Activity Center in which a variety of effects are produced by turning knobs, spinning wheels or pushing levers. For 12 month olds, the infants are presented with a Surprise Box consisting of a series of levers, buttons and slides which release small spring-loaded figures when they are operated. Each task is presented for three minutes and is videotaped for later scoring. Both frequency and duration of behaviors such as attempting to complete the task, strategies used to explore the objects, amount of exploratory behavior and the type of affect accompanying the tasks are coded.

Observations of parent-infant interaction: For this study we have developed an observational system which attempts to preserve the sequence of interaction between the parent and infant. The observer focuses on the dyad with reference to communicative behaviors, caretaking, and the object-related behaviors of each member. These observations are made without the observer having any knowledge of the information obtained from the Mastery Assessment or the Bayley Scales. In order to establish reliability in observation of parent-infant interaction, observations are made by two observers simultaneously at regular intervals during the study.

The observers use an electronic digital recording device (Datamyte, Model 901-32, Electro/General Corporation, Minnetonka, Minnesota) consisting of a small 14 button keyboard through which the coded data are entered and stored in the unit along with the time of each data entry.

Major Findings: Preliminary analyses from a cross-sectional pilot study indicate that, although there are predictable differences in the types of behaviors exhibited by 6- and 12-month-old infants on the mastery tasks, the three components of mastery motivation seem to be appropriate for both ages. We were able to construct problem solving, effectance production and sensorimotor skill tasks which are appropriate for each age. One of the consistent differences between 6- and 12-month-olds is that the younger infants are more dependent on the examiner and the mother during the task.

Data collection for the longitudinal study is virtually completed. Presently, we are analyzing interobserver reliability for the home observers. The preliminary results of reliability analyses show that the overall reliability for duration of individual behaviors averages r=.84. The estimate of Cohen's kappa is K=.799. This is impressive since the observational system is quite complex with a complexity ratio of C=.066. Interobserver agreement estimates for the observers who are coding the videotapes of mastery motivation is also at acceptable levels. The coding of these videotapes will be completed by the end of the current fiscal year, at which time data reduction and analysis of the data will begin.

Procedures for analysis of the data from both the mastery motivation assessment and the observations of parent-infant interaction are now being refined. Preliminary data reduction of the six-month observations is completed and these data await further data processing steps.

Thirty families in this study have been participants in another study on the psychological aspects of childbearing (Z01 HD 00014). The overlap in the samples from the two studies will allow us to examine the relationships between prenatal factors related to childbearing and family dynamics and parent-infant interaction at 6 and 12 months of age.

Significance for Biomedical Research and the Program of the Institute: The once prevalent assumption that all behavior is motivated by simple need reduction has shifted to the view that a striving for competence is also a powerful motivation. R. W. White (1959) contends that activities in the service of effectance are adaptive; they help the child acquire knowledge and skills. In the ensuing years there have been a number of studies of competence, effectance, and mastery motivation in preschool children (Bronson, 1973; Harter, 1976; Harter and Zigler, 1974) and some theoretical elaboration of the concept (Hunt, 1965), but there has been little progress in operationalizing the construct or developing measures to investigate the beginnings of mastery behavior in infancy. It is hoped that an understanding of the roots of mastery and of optimal patterns of parent-infant interaction can lead to more effective intervention programs.

Proposed Course of Project: The data collection will be completed by the fall of 1979. Further plans for data reduction will be developed and further data analyses will continue until July 1980. During that period, several reports of the results of the project will be written and submitted for publication.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00013-03 CFR

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Role of Mother-Infant Relationship in Subsequent Socio-emotional Development

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. P. Klein	Research Psychologist	CFR, NICHD
OTHER:	J. D. Suwalsky	Research Psychologist	CFR, NICHD
	M. W. Fivel	Research Psychologist	CFR, NICHD
	M. E. McCarthy	Research Psychologist	CFR, NICHD
	N. F. Gist	Research Psychologist	CFR, NICHD

COOPERATING UNITS (if any)

None

LAB/BRANCH
Child and Family Research Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Md. 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.10	1.95	.15

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to identify factors in infancy which are predictive of later socio-emotional development. This project will focus primarily on the aspects of the mother-infant relationship and infant functioning which are hypothesized to be important for healthy emotional development during early childhood. An earlier study of this project found that the infant's preference for mother was predictive of better social functioning at four years of age. We are currently conducting a second study in order to replicate and extend this finding.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To identify factors in infancy which are predictive of later socio-emotional development. This project will focus on aspects of the mother-infant relationship and infant functioning which have been hypothesized to be important for healthy emotional development during early childhood.

Methods Employed: The first study of this project obtained data on the current socio-emotional development of 3-4 year old children (Project Z01 HD 00011 SBS) who were originally seen at one year (Projects Z01 HD 00005 and Z01 HD 00008). We devised a Social Functioning Index on the basis of direct observation in two testing sessions, a free play session with a peer and two questionnaires--the Kohn Preschool Social Competence Scale, filled out by the child's nursery school teacher, and the Louisville Home Behavior Checklist, filled out by the child's parents. This index was a measure of adequacy of functioning in a wide variety of areas at preschool age. Our measure at one year of the mother-infant relationship was based on the infant's behavior in our Differential Social Reaction Procedure. This procedure scores the responses of the babies to a three minute, three step semi-structured play sequence, first with an unfamiliar adult then with the mother. On the basis of their reaction, we classified babies into two major groups: those who did not show clear preference for mother and those who did show a preference. We considered the presence or absence of preference for mother in this situation to be indicative of the expectations the infants had formed about their mother. (The empirical basis for this assumption was presented in the annual report for fiscal 1977.)

Major Findings: Preference for mother during the six minute play episode at one year proved to be predictive of better social functioning during preschool, $r=.49$, $p<.01$. Considering that infancy measures in the past have shown virtually no predictions to later behavior, this result takes on a particular importance. Furthermore, preference for mother at one year predicted intellectual functioning at 3 1/2 years as measured by the McCarthy Scales of Children's Abilities, $r=.37$, $p<.05$. In contrast the correlation between the Bayley Scales of Mental Development, also administered at one year, and the McCarthy Scales did not even approach significance.

Significance to Biomedical Research and the Program of the Institute: On the basis of experience with emotionally disturbed individuals, clinicians from several different theoretical perspectives have affirmed that the early mother-infant relationship is crucial for later emotional and cognitive development. There has, however, been little consensus on how to assess this relationship meaningfully. Moreover, there have been few prospective studies of the long term significance of variations in the mother-infant relationship. In this study we have found that preferential response to mother at one year was significantly related to social functioning 2 1/2 years later.

Proposed Course of Project: The findings of this study clearly require replication. To that end we have begun a second study, which involves

the assessment of the bond between mother and infant at the beginning of the second year. The development of these children through their fifth birthday will be periodically assessed by questionnaires mailed to parents and preschool teachers, and by direct observation if sufficient staff is available. In our initial assessment approximately 30 toddlers have been studied. We plan to see approximately 100 more children during the next two years. These children will be recruited from several other studies going on in the Branch which are collecting data during the first year of life. Although this strategy prolongs the period of data collection, it will provide us with valuable data on the earlier experiences of our subjects.

Publications:

Klein, R. P. and Durfee, J. T. Prediction of preschool social behavior from socio-emotional development at one year. Child Psychiatry and Human Development, 1979, 9, 145-151.

Klein, R. P., and Durfee, J. T. Comparison of attachment behaviors in home and laboratory. Psychological Reports, 1979, 44, 1059-1064.

Presentations to Professional Groups:

Klein, R. P. Role of the mother-infant relationship in subsequent social-emotional development. University of Washington, Child Development and Mental Retardation Center, July, 1979.

Klein, R. P. Parenting. Bethesda Health Center. In-Service Training, March, 1979.

Klein, R. P. Infant attachment and later socio-emotional development. George Washington University, Department of Psychology, January, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00014-03 CFR

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Psychological Aspects of Childbearing and the Neonatal Period

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K. Standley	Research Psychologist	CFR, NICHD
OTHER:	J. Nicholson	Research Psychologist	CFR, NICHD
	N. F. Gist	Research Psychologist	CFR, NICHD
	F. van der Waals	Visiting Fellow	CFR, NICHD

COOPERATING UNITS (if any)
Department of obstetrics and Gynecology, The Washington Hospital Center, Washington, D.C.

LAB/BRANCH
Child and Family Research Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Md. 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.50	2.25	.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this research program is to gain understanding of psychological aspects of the childbirth experience and their impact on the well-being of the child, the new parents, and the family unit. Childbirth is a special interaction of physiology and psychology which may have important consequences in infant development.

The current goals of this research are to develop instruments which allow for objective, comprehensive measurement of childbirth events and to describe the varieties of normal childbearing. A method has been developed for recording the physical state of the woman in labor and medical and social interactions with her. A compatible measure of delivery room events is now being constructed. Prenatal and postpartum interviews with parents provide data on their expectations and recollections of the birth which can be compared with the researcher's observations. Parental views of the newborn are contrasted with the researcher's assessment of infant behaviors

Project Description:

Objectives: Childbirth represents a special interaction of physiological and psychological events which may have important consequences for the development of the infant. The objective of this research is to increase our understanding of the psychological aspects of the childbirth experience and its impact on the well-being of the child, the new parents, and the family unit. The goals of this project are to develop instruments for objective comprehensive measurement of childbirth events.

Methods Employed: Comprehensive perinatal data have been obtained from forty middle-income, Caucasian couples and their first born infants. Data collection has been completed and analyses are underway.

Data were obtained at three points in the perinatal period. In the last month of pregnancy, the expectant mother was interviewed individually about her pregnancy experience and her expectations of birth and parenting. Demographic and historic data were also obtained.

During each woman's labor, a member of the research staff observed the woman and her husband for one hour and recorded labor room events in accordance with a time-sampling method developed for this purpose. The observation system taps various dimensions of the woman's physical state and all medical and social events which impinge on her. The observer watches the woman and others for 30 seconds and then records the observation for 30 seconds. The system has proved to be a precise and comprehensive record of the labor room experience. Hospital record information was also acquired.

One week after the birth, another member of the research staff visited the new parents in their home and interviewed them about their recollections of the birth and their new parenting experiences. These data are being compared with the researcher's actual observations of the labor. Parents also completed the Newborn Behavior Inventory, a measure of impressions of the baby's temperamental characteristics. The NBI was developed in the course of this project as a measure of parental perceptions of infants which did not depend on administration of a test to the child. The dimensions of the NBI correspond to the Neonatal Behavioral Assessment Scale which was independently given by a trained examiner. As with the measures of the childbirth setting, there are two views of the infant which can be compared--the parent's and the researcher's.

Major Findings: Several measures of childbirth events have been developed which provide objective and subjective data on prenatal, birth, and postnatal occurrences and experiences. Special attention is also being given to the early parenting experience and perceptions of the newborn.

Analyses of the objective, observational data on labor indicate significant associations between the woman's physical pain and tension and specific behaviors of her husband. Of special note is the proximity of the husband to his wife. Physical closeness to the woman in labor and certain events such as conversing and touching, may reduce her distress. We are currently

attempting to identify "styles" of interaction which couples characteristicly use to cope with the stress of labor. For example, some couples communicate clearly about the course of labor and their efforts to control discomfort, while the involvement of other couples is mediated by medical procedures and instruments, especially the electronic fetal monitor. Still other couples have limited communication and are physically distant from each other, but they may be coping effectively with stress in the manner that is most satisfactory for them.

Preliminary analyses of data on the Newborn Behavior Inventory shows significant differences in parental and examiner perceptions of the infants on the Newborn Behavior Inventory. The examiners rated the infants as more irritable and excitable and more physically responsive to stress. There are also some interesting differences in the perceptions of the two parents. Fathers, for example, seem to have an especiially positive view of the interactive behaviors of the infants as well as seeing them as more motorically mature and facile.

Further analyses will explore each measure in more depth and will focus on interrelationships among the several measures of the prenatal, birth, and postpartum periods.

Significance to Biomedical Research and the Program of the Institute: Little is known about the psychological impact of specific birth events on parents' perceptions of their experiences and on their interactions with their infants. This project and continuing research in this area will provide information on the psychology of birth which will be important to the medical practitioner and parents in their understanding of the psychological as well as physical aspects of birth events.

Proposed Course of the Project: Data collection has been completed. Analysis of the data is underway. Several papers will be presented during the next year at the meetings of the American Public Health Association and at the Association for the Advancement of Behavior Therapy.

Publications:

Anderson, B. J. and Standley, K. The newborn behavior inventory: A method for assessing parents' perceptions of their infants along dimensions of the Neonatal Behavioral Assessment Scale. JSAS Catalog of Selected Documents in Psychology, 1979, 9, 7.

Papers Presented at Professional Meetings:

Gist, N. F., Nicholson, J., Standley, K., and Zaslow, M. Obstetrical procedures and practices: Behavioral outcomes. Presented at the Society for Research in Child Development Biennial Meeting, San Francisco, Ca., March, 1979.

Nicholson, J. A model of maternal coping with childbirth: Implications for research and methodology. Presented in a symposium on "Parenthood: Coping with a critical psychophysiological life event." Presented at the Annual Meetings of the American Psychological Association, Toronto, Ontario, August, 1978.

Nicholson, J., and Standley, K. A method for naturalistic observation of the childbirth environment: With application to theory building and research. Theory Development and Methods Workshop, National Council on Family Relations Annual Meeting, Philadelphia, Pa., October, 1978.

Standley, K. Objective observation of labor and delivery. Presented at the American Society for Psychoprophylaxis in Obstetrics Annual Meeting, Colorado, August, 1978.

Standley, K. Psychosocial predictors during pregnancy of obstetrical conditions and events. Presented in a symposium on "Parenthood: Coping with a critical psychophysiological life event," Annual Meetings of the American Psychological Association, Toronto, Ontario, August, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00015-02 CFR

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Parent-Infant Interaction Subsequent to Cesarean Delivery

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. A. Pedersen	Research Psychologist	CFR, NICHD
	M. J. Zaslow	Staff Fellow	CFR, NICHD
OTHER:	R. L. Cain, Jr.	Research Assistant	CFR, NICHD
	J. T. D. Suwalsky	Research Psychologist	CFR, NICHD
	M. W. Fivel	Research Psychologist	CFR, NICHD
	E. Kramer	Student Scientist	CFR, NICHD

COOPERATING UNITS (if any)

Parent and Child, Childbirth Education Associates

LAB/BRANCH

Child and Family Research Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.3	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this study is to examine the effects of cesarean childbirth on family interaction and on the parent-infant relationship. The sample consists of 48 families with medically normal, first born infants, half having had a cesarean delivery and half a vaginal delivery. When the babies are three months old, and again when they are a year old, two observations are made of mother, father and baby interacting, and one observation is made of mother and baby alone. At each age parents are interviewed, asked to complete a Q-sort assessing their perceptions of their baby's temperament, and to record their participation in household and childcare tasks on a weekday and a day on the weekend. Cross-sectional and longitudinal analyses will assess differences in family interaction in parents' reports of their experiences during childbirth and in their adjustment to parenthood subsequent to different birth experiences.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Although the rate of cesarean delivery among middle-class mothers has more than doubled in the past decade, we have little understanding of the psychological impact of this experience on families. The purpose of the present investigation is to obtain longitudinal data on the mother-baby, father-baby and spouse relationships in families whose first baby was delivered abdominally. Comparisons will be made with families in which a normal vaginal delivery occurred. Two major questions will be addressed: (1) Does cesarean birth have significant effects on any aspect of the parent-infant or spouse relationships? (2) If there are differences, do they persist throughout the infant's first year?

Methods Employed: The sample consists of 24 families with normal, cesarean-delivered infants and 24 families with normal vaginally delivered infants. The comparison group is drawn from another study (Z01 HD 00016 CFR). The groups are matched on mother's employment status and sex of infant.

Observational procedures from a previous study (Z01 HD 00003 CFR) of mother-infant, father-infant, and mother-father interaction have been revised for use in the present study. In addition an extensive parent interview has been developed which addresses the following areas: (1) the birth experience and adjustment to parenthood; (2) division of household and child care tasks and (3) employment history, attitudes and plans. A previously developed method for studying parents' perceptions of their babies' temperament has been revised and extended for the present study. A time log has been developed to record each parent's participation in household and child care tasks on a weekday and a day on the weekend. Finally, an interview has been developed to determine the extent of mother-infant separations and arrangements for substitute care over the infant's first year.

Families are seen when the baby is 3 months old and again when the baby is 12 months old. At each age there are 2 observations of mother, father, and baby together, and one observation of mother and baby. At each age parents are interviewed, asked to complete the perception of infant temperament measure and the time log. When the infants are 6, 9, and 12 months old the interview concerning mother-infant separations is carried out by telephone.

Major Findings: The psychometric properties of the revised Perception of Baby Temperament measure have been assessed. Thirty-eight mothers with infants of between 3 and 5 months completed the Q-sort task. Split-half reliabilities and interscale correlations were computed for 5 temperament dimensions: positive mood rhythmicity, activity, approach and adaptability. Split half reliabilities show a range of .52 to .79 and a median of .68. Interscale correlations show that the scales are essentially independent with a median of .01, and range of -.31 to +.29.

Significance to Biomedical Research and the Program of the Institute: A cesarean childbirth is not only a medical procedure, but an experience that may have important psychological sequelae. Post-hoc analyses from a pre-

vious study (Z01 HD 00003 CFR) contrasting families that had had cesarean and vaginal deliveries, suggest that couples who have had a cesarean delivery experience more stress at the time of the birth and more difficulty adjusting to being parents. These parents may establish different patterns of care and interaction with their baby. The present study will attempt to clarify the distinctive experiences of families following a cesarean childbirth, and try to determine whether differences continue throughout the infant's first year. The study will help identify special needs of mothers and fathers following a cesarean delivery.

Proposed Course of Project: Measures of reliability have been completed The median reliability of observation categories was .95. Two interviewers were in exact agreement on 98.5% of the items. Observations and interviews at the first age point are presently being carried out and will continue through this spring. Training is presently under way for the 12 month observations, and the 12 month interview is being developed.

Papers Presented at Professional Meetings

Zaslow, M. Psychological impact on families of cesarean delivery. Presented at the Meetings of the Society for Research in Child Development, San Francisco, Ca., March, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00016-02 CFR

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Integration of Child Care and Work Roles

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. A. Pedersen	Research Psychologist	CFR, NICHD
	M. J. Zaslow	Staff Fellow	CFR, NICHD
OTHER:	R. L. Cain Jr.	Research Assistant	CFR, NICHD
	J. T. D. Suwalsky	Research Psychologist	CFR, NICHD
	M. W. Fivel	Research Psychologist	CFR, NICHD
	E. Kramer	Student Scientist	CFR, NICHD

COOPERATING UNITS (if any)

Parent and Child, Childbirth Education Associates

LAB/BRANCH

Child and Family Research Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.75	3.50	.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this study is to obtain longitudinal descriptive data on the experiences of families with first born infants. Three groups of families will be studied: those in which the mother plans to be the full time caregiver for the infant during its first year; families in which the mother plans to be employed before the baby's first birthday, but is not employed when the baby is 3 months old; and families in which the mother is employed by the infant's 3 month birthday. At 3 months and again at 12 months, two observations are carried out in the families' homes when mother, father and baby are together and one observation is carried out when mother and baby are alone. Parents are also interviewed, asked to complete a Q-sort to measure their perceptions of their infant's temperament, and asked to complete a time log of a weekdays' and weekend-day's participation in household and child care tasks. Data analysis will consist of cross-sectional and longitudinal comparisons of the three groups.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Mothers with very young children are being employed in unprecedented numbers. This study attempts to address a number of questions about mothers' employment decisions in families with first born infants:

1. What factors are related to mothers' decisions to seek employment or to be full-time caregivers of their infants?

2. Are there systematic differences in the behavior of mothers who are already employed when the infant is 3 months old,, mothers who expect to be employed by their babies' first birthday, and mothers who plan to provide fulll time child care over the same period of time?

3. Is the father-infant relationship affected by variation in the mother's employment role?

4. Do mother's and father's perceptions of their baby's temperament differ in families in which the mothers make different employment decisions?

Methods Employed: The sample will consist of 36 families with first born infants: 12 in which the mother is employed when the baby is 3 months old; 12 in which the mother plans to be employed before the baby's first birthday, but is not yet employed when the baby is 3 months old; and 12 in which the mother plans to be the baby's caregiver throughout its first year. Families will be seen when the baby is 3 months old and again when the baby is 12 months old.

Observational procedures from a previous study (Z01 HD 00003-05 SBS) which permit descriptions of mother-infant, father-infant, and mother-father interaction, have been revised for use in the present study. A parental interview has been developed which addresses several topics: the experience of becoming a parent; the division of child care and household tasks between mother and father; employment history, plans and attitudes of the mother and father. A previously developed method for studying parents' perceptions of their baby's temperament has been revised and extended for the present study. A time log has been developed to record each parent's participation in household and child care tasks on a weekday and a day on the weekend. Finally, an interview has been developed to determine the extent of mother-infant separations and arrangements for substitute care over the infant's first year.

Major Findings: The psychometric properties of the revised Perception of Baby Temperament measure have been assessed. Thirty-eight mothers with infants of between 3 and 5 months completed the Q-sort task. Split-half reliabilities and interscale correlations were computed for 5 temperament dimensions: positive mood, rhythmicity, activity, approach and adaptability. Split half reliabilities show a range of .52 to .79 and a median of .68. Interscale correlations show a median of .01 with a range of -.31 to +.29. None of the scales is significantly correlated with another. This measure of parental perception of baby temperament has been widely requested for use by other researchers.

Significance to Biomedical Research and the Program of the Institute: This study will provide insight into how families with young infants reach decisions about maternal employment, and how maternal employment influences interactions of both parents with an infant. These findings will help assess the impact on families of mothers' employment decisions.

Proposed Course of Project: Measures of reliability have been completed. The median reliability on observation categories was .95. Agreement on interview items occurred 98.5% of the time. Observations and interviews at the first age point are presently being carried out and will continue through this spring. Training is presently under way for the 12 month observations, and the 12 month interview is being developed.

Publications:

Pedersen, F. A., Yarrow, L. J., Anderson, B. J., and Cain, R. L. Conceptualization of father influences in the infancy period. In M. Lewis and L. Rosenblum (Eds.), The child and its family. New York: Plenum Press, 1979, pp. 45-66.

Papers Presented at Professional Meetings:

Pedersen, F. A., Zaslow, M., and Cain. R. L. Family reorganization associated with maternal employment during infancy. Presented at the meetings of the International Society for the Study of Behavioral Development, Lund, Sweden, June, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00017-02 CFR

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Cross-Cultural Comparison of Caregiver-Infant Interactions Using Film Records: Pilot Phase

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. M. Vietze	Senior Staff Fellow	CFR, NICHD
	M. J. Zaslow	Staff Fellow	CFR, NICHD
OTHER:	A. Sostek	Asst. Prof. of Psychology in Pediatrics	Georgetown University
	L. Kreiss	Anthropologist	NINCDS
	F. van der Waals	Visiting Fellow	CFR, NICHD

COOPERATING UNITS (if any)
National Institute of Neurological and Communicative Disorders and Stroke, NIH and Georgetown University

LAB/BRANCH
Child and Family Research Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.25	.25	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
This project examines the usefulness of cross-cultural films from existing archives for studying the nature of caregiver-infant interactions in different cultures. Sixteen sequences of caregiver-infant interaction from films of Fais, an island in Micronesia, have been identified for pilot analysis. A system for continuous coding of non-verbal social behaviors of caregivers and infants has been developed. These films will be compared with films of American caregivers and infants of approximately the same ages in public settings.

NIH-0040
(Rev. 10-76)

Project Description:

Objectives: The major objectives of this study are:

1. To develop a procedure for continuous coding of caregiver-infant behaviors that can be used with film or videotapes in cross-cultural studies;

2. To examine the usefulness of films made in a number of cultures as sources of data for the study of caregiver-infant interaction;

3. To examine the generality of patterns of non-verbal social behaviors of caregivers and infants across cultures.

Methods Employed: Based on observational systems previously used with digital recording devices, a method has been developed to describe non-verbal social behaviors of infants and caregivers using film records. Sixteen segments, each of at least one minute in length, have been selected from a file record taken by a visual anthropologist (D. Rubenstein) on the island of Fais in Micronesia. Films of infants under 2 1/2 years of age in proximity to a caregiver were studied. The recorded behaviors included categories of infant visual and manual activity, posture, facial expression, and the varieties of stimulation provided by the caregiver. In addition, a number of interactive behaviors are recorded, e.g., mutual visual regard and play.

In order to provide comparison data, films of American infants and their caregiver have been coded using the same procedure. Both the Fais and the American films are of infant-caregiver behaviors in public places.

Major Findings: Both sets of films have been coded, the data reduced and preliminary analyses completed. We predicted that caregivers in Fais would be less expressive than American caregivers for visually detected behaviors. Results for direction of gaze between caregiver and infant provided partial support for this hypothesis although those for facial expression showed no differences in the two cultures. We also expected the caregivers on Fais to show greater manual and contact behaviors directed toward infants than those in the U. S. This prediction has borne out though the results were not always statistically reliable. Finally, we expected that for Fais, where adoption of infants is a common practice even when the biological parents are present, the biological parents would show more restraint in interactions with their infants than other caregivers. Analyses for five behaviors supported this hypotheses. Biological parents exceeded other caregivers in only one behavior: feeding. Thus, the descriptions of ethnographers of restraint in the behavior of biological parents on Fais toward their sons and daughters was supported.

One of the major objectives of this pilot investigation was to examine the feasibility of using ethnographic film libraries as sources for observational studies of non-western cultures. This would be especially important in studying cultures which have undergone transitions but for which film records exist. Our findings suggest that this is a viable technique for

studying non-western or primitive cultures, when there are extensive supporting field notes to document information about the subjects filmed, and when the ethnographers' filming strategy is known.

Significance to Biomedical Research and the Program of the Institute: Theories of early social development point to the importance of a close, stimulating and warm relationship between infant and caregiver. However, much of the empirical support for these theories has been obtained in Western cultures. From enthnographies and from informal viewing of cross-cultural films in the archives, it is clear that in many cultures infants have multiple caregivers, that stimulation of infants is thought to be important, and that infants are not viewed as social partners. This investigation is a feasibility study for a long range project to examine cross-cultural differences in patterns of caregiver-infant interaction. It is aimed at determining whether films can be used to study this kind of fine-grained behavior.

Proposed Course of Study: Present results have been presented at one scientific meeting and this paper is being prepared for publication. Further analyses and reports are being planned. The next step is to apply the technique to other film records and to plan filming in another culture. Shortly, one of the investigators (Kreiss) will be commencing fieldwork in Papua, New Guinea. During the course of her fieldwork, she will film 64 mother-infant dyads varying in level of urbanization and maternal employment. When these films are processed, the research team will code the films using the technique developed in this pilot phase.

Papers Presented at Professional Meetings:

Zaslow, M., Sostek, A. M., Vietze, P., Kreiss,, L., van der Waals, F., and Rubenstein, D. Cross-cultural differences in expressiveness in caregiver-infant interaction: An analysis of filmed behavior on the Micronesian island of Fais and in the United States. Presented at the meetings of the Society for Cross-cultural Research, Charlottesville, Va., February, 1979.

...TITUTIONAL SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00018-02 CFR

PERIOD COVERED
October 1, 1978 to March 31, 1979

TITLE OF PROJECT (80 characters or less)

Imitation of Adult Facial Gestures in Newborns

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. M. Vietze	Senior Staff Fellow	CFR, NICHD
	B. C. Marcus	Research Psychologist	CFR, NICHD
OTHER:	A. B. Hollenbeck	Asst. Prof. of Psychology	George Mason Univ.
	A. Sostek	Asst. Prof. of Psychology in Pediatrics	Georgetown University
	J. B. Scanlon	Chief of Obstetrics	Columbia Hospital for Women

COOPERATING UNITS (if any)

Columbia Hospital for Women

LAB/BRANCH
Child and Family Research Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.25	.20	.05

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study investigated the imitative competence of newborns. In the past, infants younger than 8 months were thought to lack the perceptual-cognitive capacity to enable them to match a modeled facial gesture with their own gesture which is not seen. Recent evidence with 2 week olds contradicts this belief. The present research further explored the possibility that newborns can imitate adult facial gestures. Results suggested no evidence of newborn imitation of facial gestures presented by adults.

...-340)
...rev. 10-...)

Project Description:

Objectives: The purpose of this research is to investigate imitative competence during the first three days of life. This study builds on a previous study (Meltzoff and Moore, 1977) which reported that infants 12-17 days old have the ability to imitate various facial and manipulative gestures. Their study suggests that neonates have an innate ability to compare a visually perceived gesture with their own unseen gesture and produce the required match. The present research seeks to examine carefully the possibility that the imitative behavior reported in the Meltzoff and Moore study was not conditioned. We hope to determine whether early imitative phenomena should be interpreted as true imitation, general arousal, or conditioned response. Attempted replications to date (e.g., Jacobsen and Kagan, 1978) have not directly addressed these critical issues.

Methods Employed: Thirty-six infants, 2 to 3 days of age were recruited from the newborn nursery at Columbia Hospital for Women.

Following a feeding, infants were taken from the newborn nursery to an adjacent room. They were placed in a padded infant chair fastened to a table facing a video camera. The camera was positioned approximately six feet from the infant and aimed so that the infant's face fills the screen.

The infant's behavior was recorded on videotape for a total of eight minutes.

Half of the infants were exposed to the gestures in the following order: tongue protrusion, mouth opening; the other half were exposed to the gestures in the reverse order. The experimental session began with the insertion of a pacifier into the infant's mouth. Infants were allowed to suck on it for 30 seconds while the experimenter presented a passive face. The pacifier was then removed and a 150-second period was timed. After this baseline period the pacifier was reinserted into the infant's mouth, and the first gesture was demonstrated until the experimenter judged that the infant had watched the gesture for 15 seconds. The experimenter then stopped gesturing, resumed a passive face and removed the pacifier. A 150-second response period was presented using the identical procedure. This procedure replicated the procedure used by Meltzoff and Moore (1977).

In the second experimental situation, a passive face replaced the tongue protrusion and mouth-opening during stimulus presentation periods. In the third experimental session, during response periods 1 and 2, the experimenter attempted to elicit the desired response. For the last situation, an inanimate object (such as a pen) replaced the adult stimulus for the entire eight minutes.

Following the collection of the data on videotapes, 10 naive judges from universities in the area were trained to judge the infant's facial expressions. They were blind regarding the study design and the experimental condition to which an infant was assigned.

Major Findings: The infants were unable to remain alert with their eyes open for the 8 minute experimental session. Some infants were awake, but cried with their eyes closed. In addition, no evidence of imitation was observed in those infants who remained in a wakeful, alert state for 4 minutes. Thus we discontinued data collection after observing 36 infants.

Significance to Biomedical Research and the Program of the Institute: Recently Melzoff and Moore (1977) have hypothesized that 2 week olds can equate their own unseen behaviors with gestures they see others perform. This reported finding is contradictory to other theories, Piaget's, for example, which propose that infants do not master this skill until at least 8 to 12 months of age. If, as Meltzoff and Moore suggest, the infant's imitative competencies are present at birth, then we must change our concepts of both the innate abilities of neonates, and the earliest learning processes used by human infants.

Proposed Course of Study: This project was discontinued. Our findings did not support the research of Meltzoff and Moore (1977). We conclude that newborns lack the perceptual-cognitive capacity to enable them to match a modeled facial gesture with their own gesture which they cannot see.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00019-01 CFR

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Mastery Motivation and Social Competence in Down's Syndrome Infants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. M. Vietze	Senior Staff Fellow	CRF, NICHD
OTHER:	M. E. McCarthy	Research Psychologist	CFR, NICHD
	S. McQuiston	Research Psychologist	CFR, NICHD
	R. H. Macturk	Research Psychologist	CFR, NICHD

COOPERATING UNITS (if any)
Children's Hospital; Children's Brain Research Clinic

LAB/BRANCH
Child and Family Research Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.85	0.75	0.10

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The major objective of this research is to study Down's syndrome infants' social competence and mastery motivation in the first year of life and to examine the relationship between the mother-infant interaction and infant's mastery behaviors. Down's syndrome infants are being studied longitudinally at 3, 6, and 8 months, and a cross-sectional sample is being studied at 12 months of age. Mastery motivation is being assessed using tasks which have been developed in this laboratory. An observational system which preserves the sequence and continuity of the transactions between mother and infant is being employed to examine the mother-infant interaction. In addition, the relationship between the infants' social and mastery behaviors and specific cognitive, temperamental, and biochemical variables is being examined.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The major purpose of this investigation is to study Down's syndrome infants' interactions with the social and physical environment in the first year of life. Specifically, Down's syndrome infants' communicative competence in social interaction and mastery motivation in interaction with inanimate objects is being examined. This project extends the study of infants' mastery motivation currently underway in this laboratory (Z01 HD 00012-02) to the Down's syndrome population. Using measures of mastery and social interactive behaviors which have been developed in this branch, the project is designed to examine the development of these behaviors in Down's syndrome infants and to specify the manner in which their development differs from that of normal infants. In addition, we are interested in examining the relationship between specific cognitive, temperamental, and biochemical (serotonin level) variables and the Down's syndrome infant's mastery and social interactive behaviors. We expect that the information gained from the study of social and cognitive-motivational development in Down's syndrome infants will be useful for the development of intervention programs.

Methods Employed: Down's syndrome infants and their parents are being recruited through two physicians in Washington, D.C. and through parent-infant education programs in the Washington, D.C. metropolitan area. Both the longitudinal and cross-sectional samples will consist of 30 Down's syndrome infants. Data on a comparable number of normal infants are also being obtained. Participants in the longitudinal phase of the study are seen in our laboratory at 3, 6, and 8 months of age. At 3 months, observations of mother-infant interaction are made, the Bayley Scales of Infant Development are administered, and the mother's perception of her infant's temperament is obtained through a Q-sort technique. At 6 months, mother-infant interaction is observed, and the infants are administered the mastery motivation tasks and the Bayley Scales of Infant Development. At 8 months, the mastery tasks and Bayley Scales are administered. Infants in the cross-sectional portion of the study visit the laboratory on three occasions at 12 months of age and are administered the mastery motivation tasks and the Bayley Scales. Both the mother-infant interaction and the infant's performance on the mastery tasks are videotaped for later coding using observational procedures which have been developed for another study (Z01 HD 00008-04). In addition to the observational data, information concerning the Down's syndrome infants' serotonin levels is being obtained from the infants' physicians in order to examine the relationship between biochemical and behavioral variables.

Major Findings: Data collection is still in progress. To date, 6 infants have been seen at three months, 7 infants at six months, 2 infants at eight months, and 4 infants at twelve months.

Significance to Biomedical Research and the Program of the Institute: Although there is a substantial body of literature on the developmentally delayed infant's physical, motor, and intellectual development, as measured by standardized developmental assessment scales, motivational factores re-

lated to the developmentally delayed child's interactions with the physical and social environment have not been studied. This project attempts to determine whether such motivational differences between nonretarded and retarded children are evident in the first year of life. In addition, this study examines the relationship between the quality of the mother-infant interaction and the Down's syndrome infant's mastery behavior. There is evidence to suggest that the interaction between the developmentally delayed infant and his/her mother may be disturbed due to the poor communicative skills of the infant. Through descriptive analysis, we hope to identify those aspects of the interaction between the Down's syndrome infant and his/her mother which may disturb both mother-infant attachment and communication and which may influence the infant's interactions with the physical environment. This knowledge will be useful in the development of intervention programs for Down's syndrome infants and their parents.

Proposed Course of Project: It is expected that data analysis will continue for one year following the completion of the data collection.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00020-01 CFR

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
The Impact of Childbirth Setting on Infant Behaviors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K. Standley	Research Psychologist	CFR, NICHD
	N. F. Gist	Research Psychologist	CFR, NICHD
OTHER:	J. Nicholson	Research Psychologist	CFR, NICHD
	F. van der Waals	Visiting Fellow	CFR, NICHD

COOPERATING UNITS (if any)
Dept. of Obstetrics & Gynecology, Washington Hospital Center, Wash., D.C.
Maternity Center Associates, Bethesda, Maryland

LAB/BRANCH
Child and Family Research Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The research project is an investigation of the importance of the childbirth environment to newborn functioning. The Neonatal Behavioral Assessment Scale will be administered to healthy, week-old infants who have been born in planned home deliveries. These infants will be compared with a group of hospital born infants drawn from a current study of hospital births ("Psychological Aspects of Childbearing and the Neonatal Period," Z01 HD 00014-03). In addition to this general comparison of the two groups, an effort will be made to identify elements of the two birth environments--such as specific obstetric practices--which may account for differences in home and hospital born infants. Of particular interest is the use of obstetric medications in relation to the behavioral and neurological functioning of the newborn.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Because of the increasing numbers of expectant parents who are electing to have planned home deliveries, this study was designed to test certain assumptions of home birth proponents about the advantages of the alternative birth setting. In particular, the behavioral characteristics of infants born at home will be compared with a comparable group of infants born in traditional hospital deliveries.

Methods Employed: The methods of this study are taken from the larger research "Psychological Aspects of Childbearing." In addition, the comparative data of the hospital born sample are taken from that study.

Two measures are employed in this study and both pertain to the behavioral characteristics of the newborn infant. The mother and father are given the Newborn Behavior Inventory when the infant is 7 to 10 days of age. This instrument records the parents' perceptions of the child and the data can be compared with the Neonatal Assessment Scale which is administered by a trained examiner.

Comparisons are made between the home born infant and two hospital samples--one sample of infants born in entirely unmedicated births and one sample whose mothers were given epidural anesthesia. The two hospital groups were specified in order to provide additional explanations of any home-hospital sample differences.

Differences between groups of infants may be attributable to factors peculiar to the settings, e.g., use of analgesic drugs in the hospital.

Major Findings: Comparison of home-birth infants with infants born in the hospital are showing some significant differences. Infants born at home seem to be significantly more motorically mature than those born in the hospital. On the other hand, infants born in the hospital scored significantly higher on self-quieting ability, hand-to-mouth facility and tonus. These analyses are continuing in more detail, examining the contribution of such hospital conditions as administration of obstetric medications, to the differences among these infants

Significance to Biomedical Research and the Program of the Institute: Home birth proponents claim numerous advantages of this setting, especially the behavioral advantage of the infants. This study addresses the issue of whether the birth setting makes a difference to the newborn? The home birth movement of health consumers is not only making home births a viable alternative in several parts of the country, but it is creating social and economic pressures for change in more traditional obstetrics.

Proposed Course of Study: Collection of data from the hospital born sample has been completed as part of "Psychological Aspects of Childbirth and the Neonatal Period." Data on the home born infants are being obtained using the Newborn Behavior Inventory and Neonatal Behavioral Assessment Scale. Data collection and analysis will take about one year.

NICHD Annual Report
October 1, 1978 through September 30, 1979
Laboratory of Biomedical Sciences
National Institute of Child Health and Human Development

The Laboratory of Biomedical Sciences investigates selected biochemical aspects of normal and pathological development.

The Section on Developmental Enzymology during the past year has continued research on the significance of phosphorylation-dephosphorylation reactions in the regulation of glycogen metabolism in mammalian muscle and trophoblast.

The study on the phosphorylation of glycogen synthase by cyclic AMP-independent kinase reveals the presence of four phosphorylation sites. The phosphorylation of these four sites proceeds in a sequential order in which the site controlling the synthase activity is phosphorylated first then followed by the three sites controlling the binding of allosteric effector, glucose-6-P. The phosphorylation of the first site greatly facilitates the phosphorylation of the other three sites. Incubation of glycogen synthase with cyclic AMP-dependent protein kinase results in the phosphorylation of two sites which are different from those phosphorylated by cyclic AMP-independent kinase. Both sites affect the synthase activity but without significant effect on the binding of the allosteric effector, glucose-6-P. The phosphorylation of these two sites proceeds initially in a random order; however, the phosphorylation of one site antagonizes the phosphorylation of the other site. The functional roles of these multiple sites have been assigned by comparing the changes in the function of the synthase with the state of phosphorylation of a site. The dephosphorylation of glycogen synthase by phosphoprotein phosphatase takes place in a sequential order in which the state of phosphorylation of a site controls the dephosphorylation of the other site. This phenomenon was observed when rabbit muscle glycogen synthase phosphorylated by cyclic AMP-dependent protein kinase was incubated with phosphoprotein phosphatase and when glycogen synthase from choriocarcinoma cells was incubated with the phosphatase from glucose-starvation cells. These studies reveal the "fine-tune" regulations of the phosphorylation and dephosphorylation reactions through mutual antagonistic and synergistic interactions between the various phosphorylation sites.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00086-09 LBS

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Developmental Enzymology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K.-P. Huang	Research Chemist	LBS	NICHD
OTHER:	Z. Ahmad	Visiting Fellow	LBS	NICHD

COOPERATING UNITS (if any)

H. Chen, RRB, NICHD; F. Huang, Dept. of Preventive Medicine and Biometrics, USUHS; J. Chou, PRB, NICHD.

LAB/BRANCH
Laboratory of Biomedical Sciences

SECTION
Section on Developmental Enzymology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Topics of current interest are: (1) the regulation of glycogen metabolism in mammalian tissues, choriocarcinoma cells, and SV-40 transformed muscle cells; and (2) elucidation of the regulations of phosphorylation and dephosphorylation of glycogen synthase by protein kinases and phosphoprotein phosphatases.

PHS-6040
(Rev. 10-76)

Project Description: Z01 HD 00086-09 LBS

Objectives: The main objectives of this project are: (1) to study the regulatory mechanisms involved in the modification of proteins through phosphorylation and dephosphorylation reactions and (2) to elucidate the mechanisms controlling the changes in the state of protein phosphorylation associated with fetal development and the diabetic.

Methods Employed: The principal methods employed are: (1) protein isolation techniques, including salt- and solvent-fractionation, gel-filtration, ion-exchange chromatography, and affinity chromatography; (2) enzyme assays, which are usually based on spectrophotometric or radiometric methods; (3) characterizations of peptides by gel electrophoresis, isoelectric focusing, and peptide mapping; and (4) tissue culture techniques, including maintenance and manipulation of cells, and transformation of cells with SV40.

Major Findings: (1) Glycogen synthase from rabbit skeletal muscle can be phosphorylated by a cyclic AMP-independent synthase kinase up to 4 mol phosphate/subunit. The phosphorylation of these four sites proceeds in a sequential order in which a site has to be phosphorylated first before the other three sites can be phosphorylated. This unique controlling mechanism for the phosphorylation reaction provides a great amplification effect of the phosphorylation "cascade" which affects the glycogen synthase activity. (2) The functional roles of the multiple phosphorylation sites of glycogen synthase phosphorylated by cyclic AMP-independent synthase kinase have been defined by comparing the kinetics of the phosphorylation of the sites with the changes in the functions of the enzyme. It was found that a site controls the binding of substrate, UDP-glucose, and three sites control the binding of the allosteric effector, glucose-6-P. (3) A technique for the identification of ^{32}P-labeled phosphopeptides has been developed. This technique involves the separation of phosphopeptides by isoelectric focusing on polyacrylamide gel and the detection of the phosphopeptides by autoradiography. This technique is simpler and more sensitive than the conventional method of peptide mapping. (4) The cyclic AMP-independent synthase kinase was found to be one of the major casein kinases in the muscle extract. This enzyme can now be readily purified by affinity chromatography on casein-Sepharose. (5) The phosphorylation of glycogen synthase by cyclic AMP-dependent protein kinase results in the incorporation of 1.2-1.5 mol phosphate/subunit. Analysis of the ^{32}P-labeled tryptic peptides reveals the presence of two phosphorylation sites. The phosphorylation of these two sites proceeds initially in a random order; however, the phosphorylation of site-1 prevents the phosphorylation of site-2 but not the vise versa. This finding adds more subtlety to the regulatory function of cyclic AMP. (6) The functional role of the two sites in glycogen synthase phosphorylated by cyclic AMP-dependent protein kinase has been defined. These two sites have the same function in determining the glucose-6-P dependency of the synthase. (7) The dephosphorylation of the two sites on glycogen synthase phosphorylated by cyclic AMP-dependent protein kinase proceeds in a sequential order in which site-2 is dephosphorylated first then followed by site-1. (8) Human placental and calf

intestinal alkaline phosphatases were found to have phosphoprotein phosphatase activity which specifically dephosphorylated the site-1 of glycogen synthase phosphorylated by cyclic AMP-dependent protein kinase. This discovery will be important in elucidating the functional role of phosphorylation sites in phosphoproteins. (9) The starvation-induced change in the state of phosphorylation of glycogen synthase from choriocarcinoma cells has been further defined. The glucose starvation facilitates the dephosphorylation of the site controlling the binding of glucose-6-P.

Significance to Biomedical Research and the Program of the Institute: Investigation on the regulation of the activity of glycogen synthase, a rate-limiting enzyme for the synthesis of glycogen, provides the understanding of the glycogen metabolism in normal and pathological development. Using glycogen synthase as a model system, which is under control by multiple-sites phosphorylation with cyclic AMP-dependent and -independent kinases, we learn more about the regulation of phosphorylation and dephosphorylation reactions -- key processes in the regulation of gene expression, cellular metabolism, differentiation, and growth. The identification of the functional roles of the various phosphorylation sites in glycogen synthase will be important in determining the specific functional change in glycogen synthase associated with fetal development and the diabetic. The study on the regulation of glycogen metabolism in trophoblast is essential for an understanding of fetal carbohydrate metabolism, which is greatly influenced by the placenta before the fetal liver has fully developed.

Proposed Course of Project: We will continue to explore the regulatory mechanism of phosphorylation and dephosphorylation of glycogen synthase and to apply the findings in the study of the metabolic regulation associated with fetal development and with the diabetic.

Publications:

1. Huang, K.-P.: Regulation of glycogen synthase activity in choriocarcinoma cells. Effect of glucose starvation on the conversion of synthase D to synthase I and the kinetic properties of the enzyme. European. J. Biochem., in press.

2. Itarte, E., and Huang, K.-P.: Purification and properties of cyclic AMP-independent glycogen synthase kinase 1 from rabbit skeletal muscle. J. Biol. Chem., in press.

3. Huang, K.-P., Lee, S.-L., and Huang, F.: Phosphorylation of rabbit skeletal muscle glycogen synthase I by a cyclic AMP-independent synthase kinase. J. Biol. Chem., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00601-02 LBS

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Development of Mammalian Metabolic Regulation: Phosphorylation of Thymus Histones

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Chiharu Nakai	Sr. Staff Fellow	LBS	NICHD
OTHER:	R.K. Kutty	Visiting Fellow	LBS	NICHD

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Biomedical Sciences

SECTION
Section on Physiological Controls

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project examines effects of hormones, drugs, and nutrients on thymus metabolism during early neonatal life. Current studies seek to (1) identify factors and enzymes which regulate the phosphorylation of histones H1 and H2B, (2) determine the effectiveness of the factors in influencing the phosphorylation reaction as a function of the age of animals, and (3) define the physiological role of histone phosphorylation in the development of thymus gland.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The main objective of this project is to determine in thymocytes: (1) mechanism underlying differential modulation of cellular metabolism by hormones and nutrients and (2) factors controlling the developmental appearance and age-related changes in the mechanism.

Methods Employed: The principal methods employed are (1) thymocyte culture, (2) general protein isolation techniques and enzyme assays, (3) electrophoresis, and (4) autoradiography.

Major Findings: We have found that histones H1 and H2B actively undergo phosphorylation in rat thymocytes in culture. The degree of phosphoryaltion of histone H2B is as high that of histone H1. This phenomenon appears to be unique to the thymocyte system, because the level of phosphorylation of histone H1 is usually much higher than that of histone H2B in other cells in culture so far studied. We have also found that the addition of butyryl cyclic AMP to the medium of cultures of rat thymocytes causes an increase in the phosphorylation of histone H1 and a decrease in that of histone H2B. This finding demonstrates, for the first time, that the extent of the phosphorylation of any histones other than histone H1 is affected by cyclic AMP in intact cells. The most striking feature is that cyclic AMP can produce a reciprocal change in the phosphorylation state of histones H1 and H2B. Since histone H2B is a better substrate than histone H1 for isolated cyclic AMP-dependent protein kinase, there must be a regulatory mechanism which not only causes one substrate to be phosphorylated but also leads to a reduction of phosphorylation of another substrate.

In order to identify specific factors for the type-specific phosphorylation of histones, we tested various pharmacological agents. We have recently found that serum thymic factor and contraceptive peptide increases the phosphorylation of histone H2B without affecting that of histone H1. This, also, is the first demonstration that the phosphorylation of H2B is stimulated in intact cells by any pharmacological agents and that this increase is not associated with changes in the phosphorylation state of histone H1. Thus, thymocytes are unique in a sense that their responses to various exogenous stimuli are diverse and highly selective in terms of the phosphorylation of specific histones.

In our attempt to characterize specificity for histone-kinase and -phosphatase reactions, we used highly purified muscle enzyme preparations and chemically defined thymus histones. Since histones exist in vivo both in free form and bound to DNA, we examined the effects of DNA and other synthetic polynucleotides on histone-kinase and -phosphatase reactions. These nucleic acids inhibited the kinase reaction, and the inhibition kinetics was largely influenced by the histone/nucleic acid ratio as well as the total concentrations of both histone and nucleic acid present in assays. In contrast, these nucleic acids exhibited a variety of effects (stimulation, inhibition, or no effect) on the phosphatase reaction, and these effects were determined by the histone/nucleic acid ratio.

Significance to Biomedical Research and the Program of the Institute:

Many studies indicate that fluctuations in the phosphorylation state of histones are correlated with changes in the cell growth. There is evidence that cyclic AMP may play a role in the regulation of gene expression through phosphorylation of histone H1. Since histone H2B, but not H1, is one of the chromatin core histones, changes in the phosphorylation state of histone H2B possibly lead to an alteration in the chromatin-nucleosome structure, which may eventually affect the activities of DNA. By testing hormones, drugs, and nutrients for effects on the extent of phosphorylation of histones H1 and H2B, we learn more about physiological roles of this modification reaction. Also, we increase our understanding of the control of acute metabolic regulation, gene expression, and cell growth by elucidating mechanisms which dictate the type-specific phosphorylation of histones. Furthermore, by using thymus as a developmental model system, we can define factors controlling the age-related changes in these regulatory mechanisms during the early stages of development.

Proposed Course of Project: We plan to continue the project as outlined. We shall determine the site and extent of phosphorylation of histones H1 and H2B isolated from thymocytes that have previously received several different physiological stimuli including hormones, drugs, and nutrients. We shall continue studies on the purification and physico-chemical characterization of thymic protein kinases which exhibit high specificity for particular histone type.

Publications: None

NICHD ANNUAL REPORT

Laboratory of Developmental Neurobiology

1 October 1976 - 30 September 1979

The Laboratory of Developmental Neurobiology utilizes a variety of biochemical, morphological and physiologic methodologies in addressing the questions of the important regulatory mechanisms involved in the normal and abnormal development of the nervous system. Cell cultures of the mammalian central nervous system (CNS) are used in electrophysiological, fine structural and immunocytochemical studies of central synaptic transmission. Biochemical and morphologic analysis of factors affecting neuronal maturation in these systems are being carried out. Alterations in gene transcription are being followed as a function of differentiation in continuous neuronal cell lines and this system is being utilized in studies of receptor synthesis and localization. The pineal gland serves as a model of transsynaptic enzyme regulation; a pharmacologic dissection of this process continues and the molecular biology of the enzyme induction is under study. Studies of the molecular mechanisms of action of nerve growth factor (NGF) has shown that two specific nuclear proteins undergo phosphorylation as a result of NGF action. The relationship between these phosphorylation reactions and other NGF effects (e.g., neurite outgrowth or enzyme induction) is under study. Cell biological studies of peptidergic cells has continued in two experimental systems; 1) the hypothalamic-neurohypophyseal system of the rat which secretes oxytocin, vasopressin and neurophysin, and 2) the neurointermeidate lobe of the toad which secretes α-MSH, ACTH and endorphin. Prohormone synthesis and processing of the prohormones to produce active peptides has been extensively analyzed in the rat system. The importance of glycosylation of prohormones in regulating proteolytic processing has been demonstrated in the toad system. The vocal communication system in squirrel monkeys has continued as an object of electrophysiologic and behavioral study. Neuronal processing of vocalizations has been extensively documented so that generalizations as to the mechanisms involved can be made. The role played by the vocalizations in affiliative behavior between squirrel monkeys is under study, with the hypothesis that some semantic content of the vocal communication system will be most likely to emerge in this context.

I. Section on Neurobiology

The section continues investigation of the mechanisms of central synaptic transmission and the development of neurons and synapses. At the molecular level we are interested in gene transcription as related to neurodifferentiation. We have compared poly(A)-messenger RNA sequences from undifferentiated neuroblastoma cells with highly differentiated cells from the same clone. Hybridization of mRNA with cDNA has demonstrated both high and low abundance classes of mRNA sequences which are peculiar to either the differentiated or undifferentiated cells. We are in the process of attempting to purify the approximately 3 high abundance, differentiation specific transcripts by the use of recombinant DNA techniques.

Cell cultures of the mouse central nervous system have been used in investigating relatively long term effects of anticonvulsant agents on neuronal development. Phenobarbital has a specific dose-dependent effect as measured by decreased numbers of large neurons surviving in the cultures and by lowered levels of cholinacetyltransferase, a neuronal marker enzyme. Barbituric acid and valproic acid, another widely used anticonvulsant agent, do not have this effect. A calcium component of the action potential in mammalian central neurons has been identified and pharmacological studies of this neurobiologically important mechanism begun. Tetanus toxin has been shown to be highly active in the cell culture system and to act at a site crucial for transmitter release but not related to either the sodium or calcium excitable mechanism.

Computer-assisted discriminant analysis of data obtained from electron micrographs of horseradish peroxidase-labeled synaptic boutons has defined a number of bouton types on the basis of fine structure. Immunocytochemical techniques adapted to the culture systems have demonstrated endogenous neuronal peptides (enkephalin, substance-P, somatostatin, neurotensin) within specific cultured neurons. Further studies are in progress on the development of this peptide immunoreactivity and on the fine strutural localization of these peptides.

Work continues on the isolation and characterization of a factor produced by cholinergic neurons which aggregates the acetylcholine receptor of cultured muscle cells. Partial purification of this factor has shown that it is a glycoprotein of approximately 150,000 daltons, and is negatively charged at neutral pH. In addition to promoting the aggregation of acetylcholine receptors, material produced by cholinergic clones also promotes the attachment of a proportion of acetylcholine receptors to the myotube cytoskeleton. We have proposed a theory of synaptogenesis in which the aggregation factor is released from the endings of a presynaptic axon and promotes the attachment of diffusely distributed postsynaptic receptors to the cytoskeleton of the postsynaptic cell, resulting in the localization of receptors at the developing synapse.

II. Section on Functional Neurochemistry

The major focus of the section continues to be the study of peptides and proteins which are involved in intercellular communication (i.e., as neurotransmitters, modulators, and 'trophic' agents), and on the cell biological mechanisms by which these molecules are synthesized, transported, and secreted.

Using the rat hypothalamo-neurohypophysial system we have identified the prohormones for oxytocin and vasopressin, and shown (by tryptic mapping experiments) that each hormone is synthesized as a common precursor for the hormones and their respective neurophysins (carrier protein). The vasopressin prohormone appears to be a glycoprotein, whereas the oxytocin prohormone does not. The conversion of prohormones to peptide products occurs in the secretory granule during axonal transport to the pituitary. Current experiments involve isolation of purified secretory granules, in order to study the proteolytic enzymes in them which are involved in prohormone conversion, and to also use these granules in experiments on the mechanism of secretion of peptides. In addition, we

have shown that rats with hereditary diabetes insipidus do not synthesize the vasopressin prohormone, and hence, the disease is a defect in biosynthesis. The study of ACTH/α-MSH and endorphin biosynthesis has been conducted using the toad neurointermediate lobe. The common precursor (31K pro-opiocortin) has been identified immunologically in pulse-chase experiments. This prohormone is also a glycoprotein and studies have been directed at the significance of the carbohydrate on the molecule. The use of the drug tunicamycin has allowed us to study the fate of the prohormone which has been synthesized but not glycosylated. We have found that the carbohydrate is not essential for biosynthesis, packaging, translocation, or secretion of the peptides, but is essential for correct processing of the prohormone. In vitro studies on glycosylated and unglycosylated pro-opiocortin have shown that the glycosylated form is stabilized against non-specific proteolysis. These data indicate the importance of post-translational modifications on the processing of prohormones.

III. Section on Intermediary Metabolism

Investigators in this Section are engaged in studies on the mechanism of action and the biological role(s) of nerve growth factor. Our intent is to understand the involvement of nerve growth factor and other growth factors in the regulation and maintenance of neuronal metabolism and development.

Previous observations on the increased phosphorylation of nuclear proteins in these ganglia have been extended. We have observed that two specific proteins contain increased amounts of radioactive phosphate after the administration of nerve growth factor either in vivo or in vitro. Although these proteins have not been completely characterized as yet, one appears to be the H_1 histone, and the other a less basic protein with a similar molecular weight but a different amino acid composition. Both proteins are chromatin-bound and both occur in the neurons of the ganglia. Tryptic digests of these proteins are in progress, and we are isolating small amounts of these proteins for use as protein kinase substrates.

A difficult question is whether these events are obligatory in the overall actions of nerve growth factor on the neurons. Within the last several months we have begun to investigate this question using TPA. TPA is a tumor promoter and has been reported to prevent, at least temporarily, the nerve growth factor-induced outgrowth of neurites from sympathetic ganglia in vitro. We have recently shown that this material will also prevent the nerve growth factor-induced increase in nuclear phosphorylation. Studies on the other intracellular responses to nerve growth factor are in progress. It is hoped that this strategy will allow the linkage of the intracellular events with their eventual end results.

We have shown that the intracellular events initiated by nerve growth factor in sympathetic neurons also take place in the PC12 clone of rat pheochromocytoma. In the PC12 only one of the two proteins whose phosphorylation is increased in the sympathetic neurons exhibit increased phosphorylation. We hope to use this clone and others to dissect some of the intracellular responses seen in the ganglia. The PC12 also responds to epidermal

growth factor. This factor is a powerful mitogen in many systems, but has not previously been found to impinge on a "neuronal" cell. When cells are treated with nerve growth factor they lose most of their response to epidermal growth factor. We have found a possible reason for this, there is a marked decrease in the specific, high-affinity receptors for epidermal growth factor in the NGF treated cells. These data lead to the hypothesis that the way, or one of the ways, in which nerve growth factor stops the division of these cells is to repress the synthesis of receptors for mitogens.

IV. Section on Neuroendocrinology

The Section on Neuroendocrinology continues to study the regulation of melatonin synthesis in the pineal gland. Pineal melatonin synthesis in the monkey (as in the rat) involves positive regulation from the supra-chiasmatic nucleus of the hypothalamus, negative control from the visual system and adrenergic regulatory mechanisms in the pineal. In the hamster similar circadian oscillators appear to control motor activity and melatonin synthesis.

The pineal gland contains a high concentration of taurine, and we have shown a rapid uptake system for taurine as well as an adrenergic receptor and cyclic AMP mediated release.

The number of adrenergic receptors and their affinity for adrenergic drugs have been measured using in vitro technique. The binding of a variety of adrenergic drugs correlates very well with their affect on cyclic AMP production. The adrenergic receptor has been shown to exhibit striking, fast desensitization.

In order to measure the actual concentration of N-acetyltransferase (NAT) as well as its enzymatic activity, we are attempting to raise antibodies against the enzyme. Purification of the enzyme 100-fold has been accomplished and attempts at further purification will utilize disulfide cross linking to a special column.

Physiological inactivation of the enzyme may involve a disulfide exchange mechanism with an endogenous disulfide. This mechanism may also regulate activity of acetyl CoA hydrolase, the enzyme which breaks down acetyl CoA. Acetyl CoA in turn is necessary for production of melatonin and also protects NAT against inactivation. Thus, a small regulatory disulfide molecule could exert a powerful negative regulatory effect in melatonin synthesis; we will be continuing investigation of this interesting possibility.

V. Section on Brain and Behavior

Drs. Symmes and Newman have continued the longterm study of brain mechanisms related to the perception of a model natural language in squirrel monkeys. Work during FY 1979 has been concerned with more sophisticated analysis of individual neuron responses to species-specific vocal stimuli. The effective stimuli are brief "trigger features" within vocalizations. More than half of the cells in primary auditory cortex have such feature detector properties. The acoustic features themselves are as effective presented alone as when imbedded in natural sounds, and are present in

varying density in all parts of the vocal repertoire so far studied. It is hoped that this new evidence will facilitate development of the first functional maps of cortical areas involved in language perception.

Studies on the acoustic structure and function of sounds used in affiliative behavior of squirrel monkeys has continued under the direction of Dr. Harriet Smith. This work is founded on the hypothesis that the most interesting (and relevant to human language) aspects of monkey vocal communciation are short range, low level sounds directed at conspecifics in approach contexts, and group exchanges are now being analyzed for semantic content.

Efforts to characterize the limits of heritability in vocal behavior have continued under primary direction of Dr. John Newman. Although efforts to produce "hybrid" squirrel monkeys in our own colony have not been highly productive, we have acquired some animals from outside sources. We have continued our collaboration with the PAHO Primate Station in Iquitos, Peru, which facility has obtained (trapped) monkeys from known, naturally formed troops. It is hoped that study of this material will permit some clarification of the extent of inherited (maternal dominance) vs. learned vocal characteristics.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00047-10 LDN

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Biochemical studies of neuronal and other cell types.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B.K. Schrier	Medical Officer	LDN NICHD
	P.G. Nelson	Chief, LDN, NICHD	LDN NICHD
	G. K. Bergey	Research Associate	LDN NICHD
OTHER:	Andrzej Piekarczyk	Polish Exchange Scientist	LDN NICHD
	Dale Warren	Bio. Lab. Tech	LDN NICHD
	Kenneth F. Swaiman	IPA	LDN NICHD
	Sandra C. Fitzgerald	Biologist	LDN NICHD

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Neurobiology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	2.5	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have continued to work on determining the characteristics of cells in cultures of spinal cord cells from fetal mice. The system may be used to assess toxicity of drugs and other additives on the cells with acute or chronic treatment. The activity of choline acetyltransferase in the cultures was markedly decreased by 2 week treatment of cultures with concentrations of phenobarbital which were within the therapeutic range. This was true whether the cells were treated beginning on the day of ploting or after 1-2 weeks in culture. With both treatment schedules there was also a decrease in the number of large (>22 μm diameter) neurons in the cultures, but no loss of dorsal root ganglion neurons. With 2 weeks recovery after treatment there was return toward normal of neuron number or enzyme activity. Barbituric acid, a related compound without anticonvulsant activity, and a non-barbiturate anti-convulsant, valproate, did not similarly depress enzyme activities in the cultures.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: We are trying to define some aspects of the regulation, particularly as regards development and reaction to injury, of neurons in the CNS.

Methods Employed: Methods of spinal cord culture preparation and maintenance, as well as electrophysiologic sampling and enzyme assays have been described in previous reports. Counting of spinal cord neurons and dorsal root ganglion neurons was accomplished by use of phase contrast microscopy of unfixed cultures. A method was developed for culturing cerebral cortical cells on feeder layers of brain fibroblasts and glial cells.

Major Findings: When cultures of spinal cord (SC) cells from fetal mice were treated with 30 to 120 μg/ml of phenobarbital for 2-6 weeks there was a marked decrease in the activity of choline acetyltransferase (CAT). The amount of the decrease was dose-related. When the cells were treated from the time of inoculation, the drug had a general toxicity to the cells as evidenced by parallel decrements of protein and DNA contents. In contrast, when the SC cell cultures were allowed to develop for 7-14 days in culture before treatment, the toxicity was more selectively against CAT-producing cells. Large SC neurons, but not dorsal root ganglion neurons, also showed a decrement in treated cultures; it was not clear whether this decrement was due to a death of large neurons or to prevention of their growth to the size required for inclusion in the count. When cultures were treated from days 14-28 and then allowed two weeks for recovery, deficits of CAT activity and SC cell number persisted, although diminished in size. We cannot rule out a persistent and permanent defect.

Neither barbituric acid, a chemical relative of phenobarbital without its CNS effects, nor valproic acid, a chemically unrelated anticonvulsant, produced a decrement in CAT activity implying some specificity of the phenobarbital effect. Phenobarbital similarly decreased CAT activity in cultures of cerebral cortex cells. Preliminary studies in which SC cells were treated with either diphenylhydantoin or diazepam were inconclusive as to their effects on CAT-producing cells, but the former treatment appeared to have caused marked changes in morphology of the neurons.

Significance to Biomedical Research and the Program of the Institute: Possible deleterious effects of a number of clinically useful neuroactive agents on nervous system development have not been fully investigated. Anticonvulsants, including the barbiturates, are relatively commonly used during pre and post-natal periods when the nervous system is rapidly developing. Out initial findings indicate that these agents may not be innocuous. The culture systems may represnet one method of evaluating various agents with regard to their long-term effects on neuronal development.

Proposed Course: (a) Pursuit of the phenobarbital toxicity, particularly as regards GABA formation and glutamic acid decarboxylase activity. (b) Analysis of other anticonvulsant compounds, particularly diphenylhydantoin, valproic acid and diazepam. (c) Development of the cortical culture system for further tests of drug effects and toxicity on the developing nervous system.

Publications:

1. Holz, R. Evidence that catecholamine transport into chromaffin vesicles is coupled to vesicle membrane potential. PNAS (USA) 75: 5190-5194, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00048-05 LDN

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Studies of transcriptional level control of neurobiologic and behavioral phenomena

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L.D. Grouse	Research Associate	LDN	NICHD
	B.K. Schrier	Medical Officer	LDN	NICHD
OTHER:	C.H. Letendre	Staff Fellow	LDN	NICHD
	P.G. Nelson	Chief, LDN, NICHD	LDN	NICHD
	D. Warren	Bio. Lab. Tech.	LDN	NICHD
	J. Grouse	Student Scientist	LDN	NICHD

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Neurobiology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.8	2.2	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have continued our studies of differentiation and development in the nervous system by analysis of poly(A)containing messenger RNA population in hybridization reactions with complementary DNAs made from them.

We have found, by recycling hybridizations of cDNAs from undifferentiated and highly differentiated mouse neuroblastoma cells, that there are mRNA sequences peculiar to both differentiation states. By kinetic analysis of hybridization curves, it appeared that there were approximately 3-4 high abundance and 320 low abundance sequences specific to differentiated cells. There appeared to be 3 high abundance and 250 low abundance messenger sequences specific to the undifferentiated cells. We have also begun to analyze for differences among the non-histone chromatin proteins from cells in both differentiation states. In addition, changes in the sequence complexity of chicken liver RNA after treatment of the animals with vitellogenin were measured.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: We are investigating the extent and control of RNA sequence diversity in some manipulable systems, with particular emphasis on development and differentiation in the nervous system. The goal is to find some specific genes whose function are essential to brain differentiation, and to elucidate regulatory processes at the level of transcription which are crucial to brain development and/or function.

Methods Employed: The methods of RNA parparation, RNA/DNA hybridization, cDNA synahteis, and chromatin purification have been described in previous reports. Recycled cDNAs were obtained by hybridization to high R_0t with heterologous poly(A)-mRNAs and purification of unreacted sequences on hydroxylapatite columsn.

Major Findings:

(a) We found qualitative differences in the acrylamide gel profiles of some fractions of non-histone chromatin proteins from differentiated and undifferentiated S20Y neuroblastoma cells. There were at least two protein bands which appeared, and two others which disappeared in differentiated cells.

(b) When cDNA from differentiated (P) neuroblastoma cells was recycled by two hybridizations to mRNA from undifferentiated (S) cells; in each cycle, the unhybridized cDNA was recovered. After this material was hybridized to high R_0t with P cell mRNA, the cDNA made double-stranded in the third cycle was found to hybridize almost exclusively to P cell mRNA. Approximately 50% of this recycled cDNA consisted of 3 or 4 high abundance sequences; the remainder consisted of about 320 low abundance sequences. A similar, but less rigorous recycling of cDNA from S cell mRNA showed that there were also sequences peculiar to the undifferentiated state. These consisted of approximately 3 high abundance and 250 low abundance sequences; again, each abundance class contained about 50% of the sequences. Since differentiated cells have ceased multiplication, some of the S cell-specific sequences may be involved with G1 and S phase functions of the cells.

Significance to Biomedical Research and the Program of the Institute: We are working toward an understanding of transcriptional controls during differentiation and development of nervous system cells, particularly neurons. We hope to map out critical developmental requirements and essential gene products for differentiation in the mammalian nervous system. We believe that this knowledge will be important in the understanding of normal and abnormal CNS development.

Proposed Course: The vitellogenin project has been completed. The work on chromatin proteins has been suspended during pursuit of the

P cell- and S cell-specific sequences. Our immediate plan is to clone the P cell cDNA and use a recycled cDNA probe to identify clones which contain high abundance P cell-specific sequences. Clones from low abundance P cell-specific sequences may also be obtained and identified. The cloning will be performed according to the NIH Guidelines on Recombinant DNA; our IMUA (Memorandum of Understanding and Agreement) has been approved. We plan similarly to purify the S cell-specific sequences as time permits. Our long and short-term plans for using these sequences include the following: (a) Examination of the nuclear RNAs of the S and P cells for presence of precursors of the sequences specific to the heterologous differentiation state. (b) Analysis of poly(A)-mRNAs from other mouse cell lines, including muscle cells and other neuroblastoma lines to determine the range of specificity. (c) An anatomical and developmental dissection of the nervous system to determine which cells have these sequences expressed in their cytoplasm and at what developmental times they appear. (d) An examination of the genes from which these sequences are derived for: (i) intervening sequences, (ii) clustering in the genome, (iii) evidences of family relationships in regulation of expression of these genes, (iv) methods of regulation of these genes. (e) Attempts to identify the proteins encoded by these sequences using hybrid arrest of translation.

Publications:

1. Grouse, J.M., and Grouse, L.D. Effects of estrogen on gene expression in rooster liver. Biochem. and Biophys. Res. Communications 86:545-551, 1979.

2. Grouse, L.D., Letendre, C.H., and Schrier, B.K., Sequence complexity and frequency distribution of poly(A)-containing messenger RNA sequences from the glioma cell line C6. J. Neurochem., in press.

3. Grouse, L.D., Schrier, B.K. and Nelson, P.G., Effect of visual experience on gene expression during the development of stimulus specificity in cat brain. Experimental Neurobiology, in press.

4. Grouse, L.D., Schrier, B.K., Letendre, C.H., and Nelson, P.G., RNA sequence complexity in central nervous system development and plasticity. In press in R.K. Hunt, ed. Current Topics in Developmental Biology.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00052-11 LDN

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Biosynthesis of polypeptides in molluscan neurosecretory cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Y.P. Loh	Senior Staff Fellow	LDN NICHD
OTHERS:	H. Gainer	Chief, Section on Functional Neurochemistry	LDN NICHD
	E. Neale	Research Physiologist	

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Functional Neurochemistry

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Molluscan ganglia contain identified neurons which are excellent model systems for the analysis of neuronal regulatory mechanisms. The principal aid of this project is to study the biosynthesis, packaging, axonal transport, and release of polypeptides from these neurosecretory cells using correlative biochemical and electron microscopic autoradiographic techniques.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To study the biosynthesis, packaging, axonal transport and release of specific polypeptides from identified, molluscan, neurosecretory cells.

Methods Employed: Convention biochemical methods for the analysis of proteins and peptides are employed. In many cases, the conventional techniques of the microgradient gel for electrophoresis. Electron microscopic autoradiogrpahy is also employed.

Major Findings: The principal sites of prohormone (or propeptide) conversion to hormone (or peptide) have been located in the identified neurons. These appear to be the inner face of the golgi body and the neurosecretory granules. This information has been obtained by correlative electron microscopic autoradiography and cell fractionation experiments.

Significance to Biomedical Research and the Program of the Institute: The main significance of this work is that we have an opportunity in this model system to examine biochemical events in single neurons (i.e., in the soma) which are not accessible in more complex nervous systems. The data and approaches are directly transferrable to vertebrate systems (see project report on vertebrate peptides). Such studies provide a firm foundation for further studies of the cell biology of neuroendocrine systems, which are intimately involved in the growth and development of organisms.

Proposed Course: This project is essentially completed. Two papers (with Dr. E. Neale) are still in preparation.

Publications:

1. Ruchel, R., Loh, Y.P. and Gainer, H.: Polyacrylamide gel electrophoresis: Principles, techniques, and micromethods. In: Lahue, R. (Ed.), Methods in Neurobiology, New York, Plenum Press, in press.

2. Loh, Y.P. and Jacklet, J.: Screening for catecholamine and neurosecretory peptide synthesis in the eyes of Aplysia californica. Comp. Biochem. Physiol., 57C:159-163, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00053-11 LDN

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Information processing in the central auditory system of mammals and birds

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.D. Newman	Physiologist	LDN NICHD
	D. Symmes	Chief, Section on Brain and Behavior	LDN NICHD

COOPERATING UNITS (if any)

Department of Primate Behavior, Max Planck Institute, Munich, Germany

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Brain and Behavior

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER X

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Single unit recordings are made from neurons in the auditory system of squirrel monkey. Species-typical vocalizations previously tape recorded are used as stimuli in a series of investigations aimed at disclosing systematic parameters of neural coding of this model language. Current emphasis is in the detailed nature of acoustic features or vocal fragments which appear prepotent in driving cortical cells.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: This project is concerned with utilizing neurophysiological and neuroanatomical techniques to study processing of complex sounds in the primate central auditory system and in other model system. Special attention is being paid to sounds used in intra-specific communication, to determine which cures are used by the brain and the animal to distinguish among the various elements in the communication repertoire.

Methods Employed and Major Findings: The studies on primary auditory cortex begun last year have been successfully pursued during FY 1979. The objectives were and are to test the hypothesis that single neurons in this area exhibit response properties which justify classifying them as detectors of biologically significant acoustic features. The differences between these studies and previous work within this project are several: First, we have changed focus from "taking inventory" of cells (characterizing general properties of the region) to in-depth study of a fraction of the total cells which can be isolated. Second, we have a specific hypothesis under test (stated operationally below) regarding the logical place of such cells in a language-encoding scheme. Third, we have advanced capabilities to delete, isolate, and alter segments of natural vocalizations used as test stimuli. Fourth, we have a more critically selected (based in part on behavioral studies conducted in prior years) and acoustically improved set of vocal stimuli.

The specific hypothesis under test is that those cortical cells exhibiting stable, vigorous, discretely timed, responses to natural vocalizations do so as a result of feature locking rather than temporal facilitation. Evidence for feature locking is that short isolated fragments of vocalizations are in fact capable of driving the cells with undiminished vigor (or even increased vigor) and that deletion of the same fragments abolishes the response. This type of evidence is consistent with a feature detector model of primary cortical function, and argues that single cells in this area do not participate in a truly semantic representation of acoustic input. Evidence for temporal facilitation is that responses are dependent on temporally extended sequences of acoustic features and are greatly weakened when sequences are altered or preceding segments deleted.

We have found little evidence favoring the temporal facilitation hypothesis; and substantial evidence favoring the feature locking hypothesis. These findings in turn lead the clearer and more testable hypotheses about the way semantic encoding may proceed; i.e., either brain areas outside of primary auditory cortex are critically involved (a view our prior work indirectly supports) or the physiological properties of the system are not represented at the level of single neuron action potentials.

Significance to Biomedical Researchand the Program of the Institute: These studies represent a rigorous and technically sophisticated effort to explore the brain mechanisms involved in a highly evolved, functinally active model language. The results have significance for understanding the development of brain mechanisms mediating communicative behavior in the lower primates, and ultimately have application in the area of normal and abnormal language function in man.

Proposed Course: To examine in depth the properties of responsive primary auditory cortex units in order to test the hypothesis that some fraction of these units may be described as feature detectors for biologically relevant acoustic fragments.

Publications:

1. Newman, J.D. and Symmes, D. Feature detection by single units in squirrel monkey auditory cortex. Exper. Brain Res. Supplement (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00054-05 LDN

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Structural and behavioral analysis of vocal communication in squirrel monkeys

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.D. Newman	Physiologist	LDN NICHD
	H. Smith	Staff Fellow	LDN NICHD
	D. Symmes	Chief, Section on Brain and Behavior	LDN NICHD

COOPERATING UNITS (if any)

Primate Breeding Station (PAHO), Iquitos, Peru
Veterinary Resouces Branch, DRS

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Brain and Behavior

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.3	1.8	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is concerned with 1) obtaining a detailed description of vocal behavior of squirrel monkeys, including the frequency of occurrence of defined microstructural features in a variety of behavioral contexts; and 2) delineating the relative contribution of genetic and environmental factors to development of normal vocal behavior. Beth feral and laboratory reared monkeys are studied, and methods employed include spectrographic analysis, manipulation of social context, and formal behavioral training.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: This project is concerned with 1) obtaining a detailed description of vocal behavior or squirrel monkeys, including the frequency of occurrence of defined microstructural features in a variety of behavioral contexts; 2) delineating the relative contribution of genetic and environment factors to development of normal vocal behavior in infant monkeys.

Methods Employed and Major Findings: The major thrust of this project during FY 79 has continued to be in the area of correlating vocalizations with affiliative behavior in social groups of squirrel monkeys. Studies on laboratory-composed groups were concluded and new subject groups were established with members of natural troops. It is hypothesized that vocal communication within a natural troop is characterized by more efficient exchange of information than is found in randomly assembled groups due to shared patterns of behavior related to their common genetic and social background. There is also reason to expect greater genetic homogeneity in the natural samples, although the extent of such difference is uncertain due to our lack of knowledge of population dynamics in free-living squirrel monkeys.

The monkeys we are studying were trapped from identified natural troops in the Peruvian Amazon by personnel of the PAHO supported Primate Breeding Station, Iquitos, Peru. Drs. Symmes and Newman supervised the process selection of animals for acquisition by our program through direct visit to the Station on October 1979.

Under the primary responsibility of Dr. Harriet Smith, the new groups of monkeys are being studied utilizing quantitative computer-assisted methods. The primary objective has been solving the problem of identifying the source of individual vocalizations in group recordings. Visual markings and observation of vocalizers is difficult in some contexts, and radiotelemetry has so far appeared unsuitable to our objectives. The method we have developed involves statistical identification post hoc of the vocalizer by application of discriminant analysis of those vocal properties which have been found to be stable. Thus individual vocalizations can be attributed with 95% accuracy in a group of five monkeys without visual marking. This method permits us to examine the place of vocal exchanges in affiliative behavior patterns, without disrupting the group, and as a byproduct clearly isolates those vocal components which are not stable within individuals. The latter are very likely involved in context-dependent vocal behavior (exchange of information other than identity) and are also actively under study.

We have continued our studies on the heritability of vocal characteristics by cross-breeding strains or subspecies of squirrel

monkeys that have known vocal differences. We have augmented our own breeding program by acquisition from elsewhere of infants of known parentage. The slowly accumulating evidence continues to point to a strongly sex-linked mode of transmission, but the story is far from complete. To date, all offspring of matings between so-called "Roman" and "Gothic" parents resemble the mother physically and vocally, but the first F1 backcross animals now available do not.

Significance to Biomedical Research and the Program of the Institute: Squirrel monkeys possess one of the most specialized and discrete systems of vocal communication below man. An account of even part of the semantic aspects of this system would provide valuable insight into the function of animal languages in general and provide an excellent model for the study of language disorders in man. Also, knowledge of the otogeny of squirrel monkey vocalizations can contribute to an understanding of normal and abnormal speech development.

Proposed Course: To continue studies of social behavior and vocalizations as outlined above, and to improve the environment for such studies by developing more natural, partially outdoor, warm weather habitats at the NIH Animal Farm, Poolesville. This work is presently handicapped by the apparent impossibility of establishing behavioral facilities free of regular disturbance at the NIH campus. We also propose to continue study of heritability of focal characteristics, both by inhouse breeding and outside acquisition.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00056-04 LDN

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Regulation of ACTH, Endorphin, and MSH synthesis and secretion.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Y.P. Loh	Senior Staff Fellow	LDN	NICHD
OTHER:	H. Gainer	Chief, Section on Functional Neurochemistry	LDN	NICHD

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Functional Neurochemistry

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	0.8	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The neurointermediate lobe of the toad, Xenopus laevis, synthesizes and secretes ACTH, MSH, and endorphin. The lobe is under inhibitory, neural control (via dopamine) from the hypothalamus. We have identified the prohormone (pro-opiocortin) in this system which gives rise to the peptide products. The predominant form has a molecular weight of 31,000 and is glycosylated. Studies on the significance of the carbohydrate on the prohormone, using an inhibitor of glycosylation, tunicamycin, has revealed the role of the carbohydrate on the glycoprotein ACTH precursor to be as a mechanism for selective post-translational cleavage to the correct peptide products.

PHS-6040
(Rev. 10-76)

Project Description

Objectives To study the regulation of biosynthesis and secretion of ACTH. endorphin and MSH in the neurointermediate lobe of the toad, Xenopus laevis.

Methods Employed Generally similar to methods described in other reports from this Section, the use of acid-urea gel electrophoresis, high performance liquid chromatography (HPLC), and RIA of ACTH and αMSH are especially relevant.

Major Findings: αMSH, ACTH, and endorphin are synthesized in the lobe from the cleavage of a 31,000 dalton glycoprotein precursor. Only the products of cleavage are released, and this release is inhibited by dopamine. Eleven forms of MSH/ACTH-like peptides have been detected in the lobe of the road, and a new melanotropic peptide has been discovered and characterized in the rat brain and pituitary. Several papers on the role of precursor glycosylation are in press, and in preparation.

Significance to Biomedical Research and the Program of the Institute: Peptides such as α- and β-MSH have been implicated in higher brain functions (e.g., avoidance learning). While MSH is clearly important for melanophore control in amphibia, no clear function has emerged for this peptide in mammals. Studies on this peptide may provide insight into its functional significance. In addition, the neurointermediate lobe is under profound control by nerve terminals (biogenic amines) from the hypothalamus, and represents an excellent model system for the study of neural regulation of release and synthesis of a peptide messenger. Recently, MSH and endorphin have been implicated in fetal development as well.

Proposed Course: To further identify the ACTH-Endorphin precursor and intermediates in the toad lobe, to characterize the structure and function of the new melanotropic peptide in rat brain, and to study the mechanisms of processing and release of MSH in the toad lobe and its inhibition by dopamine. Probably the most important directions we will follow is 1) to understand the signal mechanisms (carbohydrate on the precursor) in relation to the proteolytic process, and 2) to examine the roles of these peptides in fetal and neonatal development (using tissue culture models as well).

Publications:

1. Loh, Y.P. and Gainer, H.: The role of glycosylation in the biosynthesis degradation, and secretion of the ACTH- β -lipotropin common precursor and its peptide products. FEBS Letters 96: 269-272, 1978.

2. Loh, Y.P.: Immunological evidence for two common precursors to corticotropins, endorphins, and melanotropins in the neurointermediate lobe of the toad pituitary. Proc. Nat. Acad. Sci., USA, 76:796-800, 1979.

3. Loh, Y.P., Zucker, L., Verspaget, H. and Wiersma Greidanus, T.B.: Melanotropic peptides: Presence in brain of normal and hypophysectomized rats and subcellularly localized in the synaptosomes. J. Neurosciences Res. 4:147-156, 1979.

4. Loh, Y.P. and Gainer, H.: The role of carbohydrate in the stabilization, processing, and packaging of the glycosylated ACTH-Endorphin common precursor in toad pituitaries. Endocrinology: in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00057-04 LDN

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Axonal proteins: Biosynthesis, transport, neuronal cytoskeleton, and glial-neuronal interactions.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. Gainer	Chief, Section on Functional Neurochemistry	LDN NICHD
OTHER:	D. Fink	Research Associate	LDN NICHD

COOPERATING UNITS (if any)

I. Tasaki and H. Pant, Laboratory of Neurophysiology, NIMH
R.J. Lasek, Case-Western Reserve University

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Functional Neurochemistry

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The use of model giant axons in the squid and crayfish has led to the formulation of the "Glial-Neuronal Protein Transfer Hypothesis." In addition, the giant axon has been a useful model for the study of 1) biochemical bases of excitability, 2) Intra-axonal Protein Phosphorylation, and 3) The regulation of axonal growth by Ca^{++}-activated proteases. The mechanisms underlying axonal transport have been studied using rat neurosecretory (hypothalamic) cells and rat sciatic nerve. These studies emphasize the diverse roles of cytoskeletal proteins (e.g., actin and tubulin) in fast vs. slow axonal transport. A novel method, using covalent labeling probes, to study axonal transport has been developed.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To study the biosynthetic origins of axoplasmic proteins, the mechanism and role of axonal transport and their functions in axonal excitability and transport.

Methods Employed: Protein biochemical techniques, autoradiography, and intraaxonal and membrane protein labeling techniques.

Major Findings: The transfer of macromolecules (i.e., proteins) between schwann cells and axons in the squid and crayfish has been unequivocally demonstrated. Covalent labeling techniques in combination with intraaxonal perfusion in the squid has revealed a sub-membrane structure (morphologically and biochemically) involved in excitability. The most recent findings have been an intra-axonal divalent cation regulated phosphorylation mechanism which selectively phorphorylates neurofilament proteins. This phosphyorylation is also regulated by excitation of the axon. In addition, we have found an intra-axonal Ca^{++} activated protease which selectively degrades neurofilament proteins, and have analysed its properties which are remarkably similar to Ca^{++}-activated proteases acting specifically on intermediate filament proteins in non-neuronal systems. Given the specificity of the proteases in each case, and yet their ability to recognize each others unique endogenous substrate, suggests a common structural conformation between all the diverse protein subunits which form intermediate filaments.

The fast transport of secretory granules by mammalian neurosecretory cells is accompanied by a co-transport of cytoskeletal proteins in a form unlike those found conveyed in slow transport. This suggests that the former proteins may be involved in the actual fast transport mechanism. We have recently developed a new approach to study retrograde axonal transport, and have uncovered a major endogenous protein which moves in the retrograde direction.

Significance of Biomedical Research and the Program of the Institute: This definitive demonstration that macromolecules can be transported from glia to neurons, represents the first example of such metabolic cooperation between cells in the nervous system, in situ. Such a mechanism may underly trophic interactions, and be a basis for inter-cellular communication during development. The Ca^{++}-activated proteases we have studied may play a major role in Wallerian degeneration, and formation of new nerve endings during regeneration and development.

Proposed Course: To study the significance of the neurofilament phosphorylation and degradation by the Ca^{++}-activated protease as basic mechanisms which regulate axonal growth and cytoskeletal turnover. To apply the new technique to study axonal transport (developed by Dr. Fink) to a wider variety of neuronal systems, in particular to axon systems in the brain.

Publications:

1. Pant, H., Terakawa, S., Baumgold, J., Tasaki, I., and Gainer, H.: Protein release from the internal surface of the squid giant axon membrane during excitation and potassium depolarization. Biochem. Biophys. Acta, 513:132-140, 1978.

2. Pant, H.C., Shecket, G., Gainer, H., and Lasek, R.J.: Neurofilament protein is phosphorylated in the squid giant axon. J. Cell Biol., 78:R23-R27, 1978.

3. Pant, H.C., Yoshioka, T., Tasaki, I., and Gainer, H.: Divalent Cation dependent phosphorylation of proteins in squid giant axon. Brain Res. 162:303-313, 1979.

4. Gainer, H.: Intercellular transfer of proteins from glial cells to axons, Trends in the Neurosciences, 1:93-97, 1978.

5. Pant, H.C., Terakawa, T., Yoshioka, T., Tasaki, I., and Gainer H.: Evidence for the utilization of extracellular [γ-^{32}P] ATP for the phosphorylation of intracellular proteins in the squid giant axon. Biochem. Biophys. Acta 582:107-114, 1979.

6. Pant, H.C., Terakawa, S., and Gainer, H.: A calcium-activated protease in squid axoplasm. J. Neurochem, 32:99-102, 1979.

7. Pant, H.C., and Gainer, H.: Properties of a calcium-activated protease in squid axoplasm which selectively degrades neurofilament proteins. J. Neurobiology, in press.

8. Lasek, R.J. and Gainer, H.: Metabolic interactions between neurons and glia. Ann. Rev. Neuroscience, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00058-04 LDN

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Biosynthesis and secretion of peptides in the vertebrate nervous system.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. Gainer	Chief, Section on Functional Neurochemistry	LDN	NICHD
OTHERS:	J. T. Russell	Visiting Fellow	LDN	NICHD

COOPERATING UNITS (if any)

M. Brownstein, Laboratory of Clinical Science, NIMH

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Functional Neurochemistry

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Using the rat hypothalamo-neurohypophysial system as a model, we have identified the common precursors (20,000 mw prohormones) of vasopressin, oxytocin, and their respective neurophysins. The peptides and neurophysin are synthesized first as a protein precursor(s) (mw, 20,000 daltons) and the enzymatic degradation of the precursor occurs intragranularly during axonal transport. Two precursors, one for vasopressin and one for oxytocin have been identified immunologically. Rats with heredity diebetes insipidus do not synthesize the vasopressin precursor. We have been able to demonstrate regulation of the biosynthesis and conversion process by relevant physiological stimuli (e.g., dehydration and lactation).

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To determine whether the neurohypophysial peptide hormones, oxytocin and vasopressin, are synthesized as prohormones (protein precursor), and to study the cellular mechanisms underlying this process. This vertebrate neurosecretory system is ideal as a model for the development of necessary techniques and approaches for the study of peptidergic neurons and systems in general. The latter represents a major long-term objective of this section.

Methods Employed: Gel electrophoresis, thin-layer chromatography, high-voltage electrophoresis, radioisotope technology, sterotaxic intracerebral micro-injection, and isolation of brain nuclei for analysis, high performance liquid chromatography (HPLC), affinity chromatography, RIA of AVP and oxytocin.

Major Findings: The prohormones for vasopressin and oxytocin have been identified and isolated (vasopressin prohormone, 20,000 daltons, pI 6.1; oxytocin prohormone, 20,000 dalton, pI 5.4). Intermediates of each prohormone during processing in the neurons have also been characterized. Rats with diabetes insipidus do not synthesize the vasopressin prohormone. Each prohormone has been unequivocally demonstrated to be a common precursor for the nonapeptide and its respective neurophysin. The vasopressin prohormone is a glycoprotein, whereas the oxytocin prohormone is not. Regulation of biosynthesis rates and conversion rates *in vivo* in response to physiological stimuli have been demonstrated.

Significance to Biomedical Research and the Program of the Institute: The finding that oxytocin and vasopressin are synthesized first as prohormones places these neuronal peptides in the same category as other peptide hormones (e.g., pro-insulin, insulin). Perhaps more significant is that the experimental paradigm first developed for molluscan identified neurons is highly effective as an approach for peptide biosynthesis in vertebrate systems. Such peptidergic neurons and systems in the vertebrate brain may have important regulatory (e.g., trophic) and integrative roles during development.

Proposed Course: To study the proteolytic enzymes, involved in the conversion of the prohormones to the hormones, and to examine whether these enzymes are associated with the secretory granule membrane. In addition, the specificity of these proteases for the conversion of diverse prohormones will be studied. Given the above background, we are now able to embark on the developmental aspect of this project (i.e., to study the *in vivo* development of hypothalamic peptidergic neuronal systems).

1. Gainer, H. and Brownstein, M. J.: Identification of the precursors to rat neurophysins. In "The Cell Biology of Neurosecretory Processes in the Hypothalamus," Eds. J. D. Vincent and C. Kordon, CNRS press, pp. 525-542, 1978.

2. Gainer, H. and Brownstein, M.J.: Electrophoretic analysis of proteins transported to the rat posterior pituitary. J. Neurochem., 30: 1509-1512, 1978.

3. Gainer, H.: Input-output relations of neurosecretory cells. In Comparative Endocrinology, P.J. Gaillard and H.H. Boer, Eds., Elsevier/North Holland press, Amsterdam,pp. 293-304, 1978.

4. Gainer, H., Russell, J.T., and Fink, D.J.: The hypothalamo-neurohypophysial system: A cell biological model for peptidergic neurons. In Brain Peptides, A.M. Gotto, ed. Elsevier/North Holland Press., in press.

5. Gainer, H. and Brownstein, M.J. Neuropeptides. In Basic Neurochemistry, R.W. Albers and G. Siegel, eds., Amer. Soc. Neurochem., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00059-04 LDN

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Studies of postsynaptic pharmacology of mammalian neurons in culture

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. K. Bergey	Research Asscociate	LDN NICHD
OTHER:	M. Martin	Staff Fellow	LNO NINCDS

COOPERATING UNITS (if any)

Laboratory of Neuro-otolaryngology, NINCDS

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Neurobiology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Using dissociated fetal mouse spinal cord cells grown in culture, we have investigated aspects of the postsynaptic pharmacology of these mammalian central neurons. In culture these neurons are known to exhibit characteristic responses to the excitatory amino acid glutamate (GLU) and the hihibitory amino acids glycine (GLY), and gamma-aminobutyric acid (GABA).

Selective inhibotors for the excitatory amino acids glutamate and aspartate have been sought. Adicarboxylic acid D-L-α-aminoadipate (DLAA) was found to markedly antagonize aspartate iontophoretic responses, while having much less effect on glutamate responses.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: We wanted to investigate aspects of the postsynaptic pharmacology of dissociated mammalian neurons in culture. Specifically, investigated were the known responses to the putative neurotransmitter amino acids and the modification of such responses by certain agents. Obtaining useful means of identifying the excitatory transmitter for known synaptic connections was of particular interest.

Methods Employed: Fetal mouse spinal cord neurons were grown in dissociated cell cultures for five to ten weeks. Using KCl or KAc micropipettes, individual neurons were penetrated and stable intracellular recordings were obtained while the culture was maintained on a heated, pH controlled stage of an inverted phase microscope. Responses to amino acids were obtained by iontophoresis through micropipettes immediately adjacent to the cell soma. Another adjacent iontophoretic pipette containing the agent under study (DLAA) was used to test for modulation of amino acid responses.

Major Findings: DL- -aminoadipate markedly antagonized postsynaptic responses to iontophoretically applied aspartate; antagonism of glutamate was much less prominane. Responses to the inhibitory amino acids GABA and glycine were unaffected by DLAA. No direct effects of DLAA on membrane potentials or passive membrane properties were seen.

Significance to Biomedical Research and the Program of the Institute: Dissociated neuronal cultures provide a system that optimizes cell accessibility and the ability to alter cellular milieu. This allows investigation of the postsynaptic mechanisms that may account for the actions of therapeutic agents (e.g. anticonvulsants) as well as potentially pathological substances. The identification of excitatory synaptic transmitters has been an area of relatively slow progress. Better tools, such as specific inhibitor like DLAA, will be important in approaching this important area.

Proposed Course: We plan to study some identified excitatory synaptic connections (between dorsal root ganglion and spinal cord cells or between two spinal cord cells) to see whether DLAA has a selective depressant effect on these synapses.

Publications:

1. Macdonald, R.L. and Barker, J.L. Enhancement of GABA-mediated postsynaptic inhibition in cultured mammalian spinal cord neurons: A common mode of anticonvulsant action. Brain Res., 167:323-336, 1979.

2. Macdonald, R.L. and Barker, J.L. Anticonvulsant and anesthetic barbiturates: Different postsynaptic actions in cultured mammalian neurons. Neurology, 29:432-449, 1979

3. Macdonald, R.L. and Barker, J.L. Valproic acid augments GABA-mediated postsynaptic inhibition in cultured mammalian neurons. Brain Res. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00062-03 LDN

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Brain mechanisms of vocal production in squirrel monkeys

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.D. Newman	Physiologist	LDN NICHD

COOPERATING UNITS (if any)

Laboratory of Brain, Evolution and Behavior, Dr. Paul MacLean, Chief
Department of Primate Behavior, Max Planck Institute, Munich, Germany

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Brain and Behavior

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.3	.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Vocalizations from adult squirrel monkeys with defined brain lesions are recorded onto magnetic tape and analyzed with the sound spectrograph. Subjects are tested and observed in a range of social contexts to characterize vocal changes following damage to specific brain structures. Current work is directed toward an assessment of the effects on vocal performance of lesions in tegmental pathways.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: This project is concerned with the brain sites and neural mechanisms by which vocalizations are initiated and controlled. A major objective is to form a link with our ongoing studies of the perceptual and behavioral aspects of vocal communication, leading to an overall understanding of the communicative process. A further goal is to correlate the brain mechanisms of vocalization with those controlling other forms of emotional behavior, to enlarge our understanding of the neural control of social behavior in general.

Methods Employed and Major Findings: Histological examination of the brains of three monkeys in our current experimental series provided new information regarding definition of tegmental regions involved in the control of vocalizations. Bilateral lesions in the vicinity of nucleus parafacicularis that severed the habenulo-peduncular tract resulted in no alteration in Isolation Peep structure or threshold of production (as measured by rate of utterance pre- and post-operatively). However, in 2 subjects with lesions involving the central tegmental tract, post-operative production of Isolation Peeps was greatly reduced. Both monkeys were vigorous producers of this call type prior to surgery, and both failed to reach pre-op levels of vocalization during the entire 9-12 month post-op study period. In the more severaly impaired monkey (M5) no peeps at all were recorded post-op, whereas other call types (errs, chucks, cackles) did occur. This subject therefore demonstrates call-specific "muting" and, together with a previous similar case, delimits the brain area involved as periaqueductal gray and adjacent tegmentum.

Significance to Biomedical Research and the Program of the Institute: Prior work on neurological intervention of mammalian vocal behavior has demonstrated either complete inability to vocalize (mutism) or inability to performed conditioned vocalizations. Results from this project are the first to demonstrate deviations from normal vocal communication following discrete lesions to the central nervous system. The future course of this work may provide more complete information about brain structures important in controlling articulation, patterning, and thresholds of vocalization. This work is therefore relevant both to the pathology of social communication as well as to the neural mechanisms controlling emotional expression.

Proposed Course: Additional monkeys are scheduled for lesions in the rostral periadequeductal gray to confirm the role of this brain region in the production of the isolation call. A wider range of contexts will be studies to enlarge the classes of vocalizations available for analysis following surgery.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00064-02 LDN

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Neurobiologic studies of neurons and glia from the mammalian central nervous system in cell cultures.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P.G. Nelson	Chief, LDN, NICHD	LDN NICHD
	E.A. Neale	Research Physiologist	LDN NICHD
OTHERS:	G. Bergey	Research Associate	LDN NICHD
	E. Matthew	Guest Worker	Columbia Univ.
	W. Habig		DBP BB

COOPERATING UNITS (if any)

Department of Neurology, Columbia University

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Neurobiology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.7	1.7	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Calcium currents have been shown to be partially responsible for electrical activity of both spinal cord and DRG cells.

Tetanus toxin applied to spinal cord cultures results in an initial increase of paroxysmal excitatory activity followed by a loss of all spontaneous synaptic activity. This generalized blockade of synaptic activity occurs in the absence of any measurable change in either sodium or calcium dependent action potentials.

Neurons in cultures of spinal cord and dorsal root ganglia have been shown by immunological staining techniques to contain a variety of neuropeptides, including substance P, enkephalin, neurotensin, and vasoactive intestinal peptide.

Discriminant analysis of synaptic ultrastructure of identified boutons has indicated that certain morphologic features are reliable predictors of cell type.

Project Description:

Objectives: To understand the mechanisms underlying central synaptic action and to study the factors affecting formation and development of central neurons and their synapses.

Method Employed: A reliable method of establishing central neurons in cell cultures has been developed. Intracellular recording and iontophoretic application of neuroactive compounds is coupled with regulating the environment of the recorded neurons by a perfusion system. Intracellular staining of nerve cells with horseradish peroxidase allows positive identification of all ramifications of the injected neurons in electron micrographs. Immunologic staining methods for a variety of peptides involving the peroxidase anti-peroxidase antibody have been adapted to the culture system.

Major findings: The rapid-onset increase in paroxysmal excitatory activity of neurons in cultures exposed to tetanus toxin is associated with a decrease in inhibitory synaptic activity. Within 6 hours of toxin exposure, neurons exhibit no spontaneous synaptic activity, although action potentials are generated upon electrical stimulation.

An attractive hypothesis for the mechanism of this blockade of synaptic transmission was that tetanus toxin might block the calcium dependent component of the action potential which is essential for synaptic transmitter release. We have demonstrated and quantitated the calcium currents that occur in both spinal cord (SC) and dorsal root ganglion (DRG) neurons; no change in these currents are produced by tetanus toxin treatments that suppress synaptic activity.

Neurons in combined SC and DRG cell cultures exhibit a full range of endogenous neuropeptides. The distribution of these peptides is altered when SC is cultured separately from DRG, and when nerve growth factor is added to the culture medium.

Multivariate statistical analysis of the ultrastructural features of boutons derived from a DRG neuron, one excitatory and one inhibitory SC neuron, labeled by intracellular injection of horseradish peroxidase, has indicated that the morphology of a given bouton type is consistent, and that morphology of the three types is sufficiently distinctive to statistically separate them.

Significance to Biomedical Research and the Program of the Institute: The Institute is directly concerned with factors that are of importance for the normal maturation and function of the nervous system. The role of neuropeptides in both these regards may be of great importance and the use of model culture systems should contribute to our understanding of that role. Neurotoxins have proven extremely valuable in specific probes of neurobiologic

function, and greater understanding of tetanus toxin is important in this regards as well as from a clinical standpoint.

Proposed Course: Further physiologic and anatomical studies of the central nervous system cell cultures are planned which involve the mechanisms of actino of neuropeptides, neurotoxins, and anticonvulsant agents. Experimental analyses will be expanded to a) study development of neuronal peptide activity and association of aspects of synaptic response with specific peptides, b) to examine whether treatment with tetanus anti-toxin after toxin exposure results in recovery of synaptic activity and, using ^{125}I-tetanus toxin, to determine whether intracellular location of the toxin correlates with the course of electrical events, c) to examine ultrastructural and electrophysiologic effects of anticonvulsant treatment, and d) to increase the sample base for statistical analysis of synaptic ultrastructure and to combine this analysis with additional morphological labeling techniques (for specific peptides, transmitters, receptors) in order to better study synaptogenesis and factors that affect this process, f) modulation of calcium currents by peptides and other pharmacological agents will be studies.

Publications:

1. Henkart, M. and Nelson, P.G. Evidence for an intracellular calcium store releasable by surface stimuli in fibroblasts (L cells). J. Gen. Physiol. 73:655-673, 1979.

2. Nelson, P.G. and Henkart, M. Oscillatory membrane potential changes in cells of mesenchymal origin: The role of an intracellular calcium regulatory system. J. Exp. Biol. In press.

3. Nelson, P.G., Neale, E.A., Macdonald, R.L. and Ransom, B.R. Chemosensitivity and synaptic activity in mammalian central nervous system cell cultures. In Iontophoresis and Transmitter Mechanism in the Mammalian Central Nervous System. Eds. Ryall and Kelly, Elsevier North-Holland Biomedical Press, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00089-05 LDN

PERIOD COVERED

October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Pineal-pituitary interactions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D.C. Klein	Physiologist	LDN NICHD
OTHER:	L. Tamarkin	Guest Worker	LDN NICHD

COOPERATING UNITS (if any)

J. Martin, Ph.D., Dept. of Pharmacology, Washington Univ. Med. School; K. Kirk, LC, NIAMMD; Kevin Catt, Reproductive Biology Branch, NICHD.

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Neuroendocrinology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Many life processes are regulated by the pituitary gland. This project studies how the pineal gland, influences pituitary function. The primary control of the pituitary gland is via the release of brain hormones. One of these hormones, luteinizing hormone releasing factor, regulates the release of a pituitary hormone necessary for normal reproduction, luteinizing hormone. A pineal secretion, melatonin, prevents the brain hormone from stimulating the release of luteinizing hormone. Similar effects are found using new synthetic derivatives of melatonin. The function of the pineal gland is drastically altered by environmental light and by drugs; pineal function also changes markedly during development. Thus it seems possible that as a result of these changes, and subsequent pineal-pituitary interactions, there will be significant changes in pituitary gland function resulting in turn in alterations in general health. Such an interaction may explain why the pineal gland can stop reproduction in certain animals when they are deprived of light.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To chemically and physiologically characterize pineal compounds which influence function of the pituitary gland, including those aspects concerned with reproduction. To describe the mechanism through which pineal compounds act and to design drugs which are more potent and long lived as compared to the parent compound, or block the effects of the pineal compounds.

Methods Employed: A pituitary organ culture system and a pituitary cell culture system using neonatal and adult pituitary tissue are the model systems studied. This approach provides a highly sensitive and precise means of measuring the effects of pineal hormones on the pituitary gland. In vivo systems are also used to further explore the effects of compounds of interest and of alterations in pineal function. Immunoassay procedures are used to measure the amount of pituitary hormones released following treatment with test compounds. The number of receptors present on the pituitary gland and on pituitary target tissues is determined using radio-receptor assays.

Major Findings: (1) It has been possible to synthesize a new derivative of melatonin, 6-methoxymelatonin. This compound was designed to prevent inactivation through hydroxyation at the six position. It has been found that the metabolism of this compound is slower than that seen with melatonin. (2) 6-Methoxymelatonin has been found to mimic the effects of melatonin in vitro, to block the effects of LHRH on LH release from the pituitary gland. (3) The effects of changes in photoperiod on the number of gonadotrophin receptors in the testis have been examined. It has been found that during a 24 hour period there is no change in the apparent number of receptors present in this tissue.

Significance to Biomedical Research and the Program of the Institute: These studies are consistent with the mission of the Institute to support research in reproductive biology. The results of these studies may lead to an increased understanding of the role of the pineal gland in reproduction and to the development of new drugs to alter reproduction by either mimicking the effects of pineal compounds or blocking their effect. Finally, abnormalities in reproductive physiology may be explained on the basis of pineal function.

Proposed Course of Project: (1) The effets of 6-methoxymelatonin on pituitary function will be further determined. In addition, the effects of this compound on the biological half-life of melatonin will also be determined. The purpose of these studies will be to determine whether or not 6-methoxy-melatonin is a valuable substitute for melatonin. (2) The effects of altered photoperiods, which normally produce reproductive quiescence, on the receptors for gonadotrophins in the testis and also on the number of receptors for LHRH in the pituitary gland will be determined. In addition, it is expected that the effects of injections of melatonin which produce gonadal atrophy, on the receptors of interest in testis and in the pituitary will be determined. The

goal of these studies is to determine the mechanism of action of the pineal gland and of melatonin on reproductive function in the Syrian hamster.

Publications:

1. Martin, J.E., McKellar, S., and Klein, D.C.: Melatonin inhibition of the in vivo pituitary response to luteinizing hormone-releasing hormone. Endocrinology, 1979 (in press).

2. Martin, J.E., Kirk, K., and Klein, D.C.: Relative effects of 6-hydroxymelatonin and 6-difluromelatonin on LH-RH-stimulated release of LH. Endocrinology, (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 0093-05 LDN

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Mechanism of Action of Nerve Growth Factor

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. Guroff	Chief, Sec. on Intermediary Metabolism	LDN NICHD
OTHERS:	M.Y. Yu	Senior Staff Fellow	LDN NICHD
	K. Huff	Research Associate	LDN NICHD
	C. Letendre	Research Chemist	LDN NICHD
	T. Ikeno	Visiting Fellow	LDN NICHD
	S. Hori	Visiting Scientist	LDN NICHD
	S. Vinores	Staff Fellow	LDN NICHD
	G. Dickens	Bio Lab Tech	LDN NICHD
	N. Tolson	Biologist	LDN NICHD
	P. Montgomery	Stride Trainee (1/2 time)	LDN NICHD

COOPERATING UNITS (if any)
Dr. Tom Lloyd, Division of Reproductive Biology, Hershey Medical Center

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Intermediary Metabolism

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
9.5	8.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this research is to provide information on the mode of action of nerve growth factor. Nerve growth factor is required for the development and maintenance of the sympathetic nervous system and may be prototypic for factors involved in differentiation of various cell types. The mechanism of action of nerve growth factor is of interest because it will lead to an understanding of the way in which gene expression is regulated in neurons and how such factors induce the synthesis of specific enzymes for the biosynthesis of the neurotransmitter, norepinephrine. In turn, such information may lead to an understanding of the development and differentiation of the non-dividing, non-replaceable neurons of the central nervous system, and to information about the tumors that arise from them.

PHS-6040
(Rev. 10-76)

Objectives: (1) To investigate the mechanism of action of nerve growth factor on the differentiation and maintenance of the sympathetic nervous system; (2) to delineate the intracellular events mediating the action of nerve growth factor in sympathetic and sensory neurons; (3) to detect and characterize other factors which direct the development of other types of neurons; (4) to study the interactions of nerve growth factor with other factors impinging on the cell, especially the mitogens; (5) to investigate the effects of nerve growth factor and its antiserum on the growth rate and characteristics of tumors of neural crest origin; (6) to characterize the action of nerve growth factor on the central nervous system, and especially on the hypothalamus; (7) to measure the levels of nerve growth factor in clinically relevant conditions, such as those involving tumors of the sympathetic nervous system or of other neural crest-derived tissues.

Methods employed: The methods include standard techniques of cell culture and *in vivo* investigations of the sympathetic nervous system. Enzyme assay, small animal surgery, and stereotaxic administration are used, as well as biological and immunological methods by which nerve growth factor is measured. Radioisotope techniques are employed with heavy emphasis on the use of protein labeling with ^{125}I and phosphorylation studies with ^{32}P. Slab gel electrophoresis provides an integral tool for the separation of nuclear proteins. Standard immunological techniques are employed for the preparation and use of the relevant antibodies.

Major findings: (1) The administration of nerve growth factor causes the phosphorylation of two specific chromatin-bound nuclear proteins in the neurons of the sympathetic ganglia; one of these is the H_1 histone and the other is similar in molecular weight but different in charge and amino acid composition; (2) nerve growth factor administration causes a rapid increase in the activity of protein kinase; (3) PC12 cells respond to epidermal growth factor, a potent mitogen, and terminal differentiation of PC12 cells by nerve growth factor leads to a loss of receptors for epidermal growth factor; (4) the addition of nerve growth factor rapidly alters the adhesiveness of F98 cells; (5) the addition of carbachol, a cholinergic receptor agonist, leads to a rapid activation of the enzyme tyrosine hydroxylase; (6) the action of nerve growth factor on the central nervous system is modulated by catecholaminergic neurons.

Significance to Biomedical Research and the Program of the Institute: Nerve growth factor is the best characterized and, indeed, the first known growth factor. It is required for the growth and maintenance of sympathetic neurons. Its mechanism of action, therefore, is of extreme interest and only the outlines are apparent. A full understanding of the mechanism of action of nerve growth factor might lead to a better understanding of the mode of regulation of the growth of neurons and of their differentiation. Further, understanding of the mechanism by which nerve growth factor affects cell development may give clues as to the normal regulation of the growth of mammalian cells and, specifically, of neurons, and to the process of genetic expression. Then, studies on nerve growth factor may indicate ways in which the selectivity of neural connectivity can be understood and, indeed, altered. A molecular

understanding of nerve growth factor action may lead to a common thread for the action of growth factors in general on their target cells. Finally, an understanding of genetic expression and growth regulation in normal cells and in the tumors which arise from them may give some new insights into tumor cell biology.

Proposed course of the project: (1) Continued study of the nuclear events following nerve growth factor action on the cell; identification and characterization of the proteins containing increased amount of phosphate; identification of the sites of phosphorylation; effect of TPA and retinoids on the phosphorylation; attempts to duplicate this effect of nerve growth factor using purified nuclei. (2) Study of the protein kinases responsive to nerve growth factor; characterization of their cAMP status; use of purified nuclear proteins as substrates. (3) Use of PC12 to fully characterize the action of epidermal growth factor on these cells; determination of the mechanism by which nerve growth factor decreases the epidermal growth factor receptors; search for a similar relationship between nerve growth factor and mitogens in normal embryonic sympathetic neurons. (4) Description of the effect of nerve growth factor on the adhesiveness of F98; isolation and characterization of the factor in conditioned media which also affects adhesiveness; detailed study of the effects of nerve growth factor and its antiserum on this tumor in vivo and in vitro. (5) Study of the mechanism by which carbachol stimulates the action of tyrosine hydroxylase in sympathetic neurons; determination of differences in kinetic behavior of treated and control enyzmes; determination of possible differences in phosphorylation. (6) Continued study of the effects of nerve growth factor in the central nervous system; determination of the possible release of pituitary peptides; inspection of alterations in behavior; search for hypothalamic activation and changes in monoamine metabolism.

Publications:

1. Guroff, G.: Phenylketonuria: Clinical and Biochmeical relationship. In Kumar, S. and Rathi, M. (eds.): Perinatal Medicine, Oxford, Pergamon Press, 1978, pp. 219-239.

2. Letendre, C.H., Nagaiah, K., and Guroff, G.: Brain amino acids. In Kumar, S. (ed.): The Biochemistry of the Brain, Oxford, Pergamon Press, in press.

3. MacDonnell, P., Huff, K., Grouse, L., and Guroff, G.: Brain nucleic acids. In Kumar, S. (ed.): The Biochemistry of the Brain, Oxford, Pergamon Press, in press.

4. Huff, K., Lakshmanan, J., and Guroff, G.: RNA polynerase activity in the superior cervical ganglia of the neonatal rat: The effect of nerve growth factor. J. Neurochem., 31: 599-606, 1978.

5. Nagaiah, K., Lakshmanan, J., Montgomery, P., Yu, M.W., and Guroff, G.: Characterisics of the purified nerve growth factor antibody. J. Neurochem., 31: 647-655, 1978.

6. Guroff, G.: Inborn errors of metabolism and the nutrition of the brain. In Wurtman, R.J. and Wurtman, J.J. (eds.): Nutrition and the Brain, Vol. 3. New York, Raven Press, in press.

7. Guroff, G.: Molecular Neurobiology, New York, Marcel Dekker, Inc., in press.

8. Huff, K.R. and Guroff, G.: Epidermal growth factor stimulates the phosphorylation of a specific nuclear protein in chick embryo epidermis. Biochem. Biophys. Res. Comm., 85, 464-472, 1978.

9. Lloyd, T., Montgomery, P., and Guroff, G.: The receptor-mediated, short-term activation of tyrosine hydroxylase in organ cultures of rat superior cervical ganglia. Biochem. Biophys. Res. Comm., 86, 1058-1062, 1979.

10. Ikeno, T. and Guroff, G.: The effect of vasopressin on the activity of ornithine decarboxylase in rat brain and liver. J. Neurochem., in press.

11. Huff, K.R. and Guroff, G.: Nerve growth factor-induced reduction in epidermal growth factor responsiveness and epidermal growth factor receptors in PC12 cells: An aspect of cell differentiation. Biochem. Biophys. Res. Commn., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00094-09 LDN

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)
Regulation of Neuroendocrine Metabolism: Circadian, Stress, Light and Drug Influences (rat, hamster, rhesus monkey)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. C. Klein	Physiologist	LDN NICHD
OTHER:	J. L. Weller	Chemist	LDN NICHD
	S. Reppert	Cilinical Associate	NPMB NICHD

COOPERATING UNITS (if any)
R.Y. Moore, Dept. of Neurosciences, Univ. of CA, School of Medicine; R. Kvetnansky, LCS, NIMH; H. Hoffman, EBR, NICHD: M. Perlow and M. Mishkind, NIMH.

LAB/BRANCH
Laboroatory of Developmental Neurobiology

SECTION
Neuroendocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to increase our understanding of the factors which regulate the metabolism of a neuroendocrine organ, the pineal gland. These investigations are conducted within a heirarchy of levels of biological organization, including the interactions of the pineal gland with environmental lighting, stress, drugs, endocrine organs, and the central nervous system. Studies related to the central nervous system also probe the fundamental nature of the biological clock governing biological rhythms.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To determine how stress, central neural structures, environmental and pharmacological factors influence horomonal secretion by the pineal gland, and to study the fundamental nature of circadian rhythms.

Methods Employed: (1) To determine the central neural, environmental and pharmacological factors influencing the pineal gland, pineal glands are removed from treated animals and the activity of metabolites and enzymes are measured. In addition, the amount of melatonin in blood, CSF and the pineal gland is measured. The animals are treated with drugs, hormones, or experimental environmental regimes, including stress and constant darkness, and in some cases are surgically prepared by the precise destruction of small areas of the brain and neural structures thought to be part of the neuronal circuit regulating the pineal gland.

In addition, small areas of the brain known to be involved in the regulation of the pineal gland are removed and the amounts of neurotransmitters they contain are determined after experimental treatment.

(2) Circadian rhythms are studied by measuring the effects of destruction of brain areas on the circadian rhythm in pineal N-acetyltransferase activity, and in melatonin in the circulation.

Major Findings: (1) It has been found that the daily rhythm in CSF melatonin in the rhesus monkey is truly circadian in nature, persisting in constant darkness for six days. In addition, it has been found that light can cause a rapid decrease in melatonin in the CSF of the primate. These effects of light are apparent throughout the night. The opposite, however, is not true: exposure of animals to darkness during the day does not cause an increase in melatonin. (2) The development of the rhythm in pineal melatonin has been studied in several species including the hamster and the rat. In both animals the rhythm in melatonin is absent at birth and developes at about the end of the second week of life. The development of this rhythm closely parallels the development of enzymatic machinery required for the last step in melatonin production, the enzyme involved being hydroxyindole-O-methyltransferase. In addition, it has been found that the photoic regulation of the melatonin rhythm in the rat is apparent as soon as the animal is capable of synthesising melatonin on a rhythmic basis. This would indicate that the neural connections between the eye and the pineal gland are complete by the end of the second week of life. (3) Analysis of the circadian rhythm in pineal melatonin in the Syrian hamster has revealed that this is truly circadian and in addition has the unique characteristic of being closely linked in time with the rhythm in running activity in this animal. Under conditions in which both rhythms are allowed to free run, such as in constant darkness, these two rhythms retain close association in time with one another, but lose a close association with solar time. (4) These studies have also revealed that the rhythm in melatonin in the hamster pituitary gland is strikingly different from the rhythm in melatonin production seen in other

animals. In the hamster the period during which melatonin production takes place is short, being no more than four hours, and exhibits a sharp peak late in the night. In contrast in the monkey, the sheep and the rat the period during which melatonin is synthesised is about twice as long. The short period during which melatonin is synthesised in the hamster makes it possible for subtle differences in the timing of the occurrance of melatonin production to be detected. Accordingly, studies aimed at determining whether or not alterations in photoperiod which alter reproductive physiology might also alter the pattern of pineal melatonin production found that the rhythm in melatonin was slightly altered and that a two hour difference in the timing of the melatonin rhythm occurred. This has rasied the possibility that the precise circadian organization of melatonin production is required for melatonin to play a reliable role in controlling seasonal reproduction. Apparently a precise relationship between pineal melatonin secretion and the sensitivity of pineal target tissues is required in seasonal control of reproduction. It would appear that subtle shifts in melatonin production might produce significant alterations in reproductive function.

This has led to a new concept of the action of melatonin on reproductive physiology that can be summarized as follows: "It's not how much, but when".

(4) The exciting new discovery has been made that the destruction of the suprachiasmatic nucleus in the primate has remarkable effects on the rhythm in CSF melatonin in the primate. Apparently in this sub-human primate as is true of the rat, the suprachiasmtic nuclei play an important role in generating the daily rhythm in melatonin.

(5) It has been found that the rhythm in melatonin in the rhesus monkey can be shifted by shifting enviornmental lighting. Interestingly, we have found that there is entrainment within a one-day period when the rhythm is shifted 12 hours by extending the normal day period twelve hours, providing the animal with a continuous period 24 hours of constant light. In contrast when an animal is provided with a extended night period, providing 24 hours of constant darkness, the period required for the animal to entrain to the new lighting cycle is three days.

Significance to Biomedical Research and the Program of the Institute: A growing body of knowledge points to the pineal gland as the source of a compound that inhibits reproduction, and that this compound is melatonin. Large changes in melatonin production are regulated by N-acetyltransferase. Thus, an increase in understanding of how melatonin production is regulated provides a more complete description of the interaction of the pineal gland in reproductive physiology.

With knowledge in hand regarding the factors which regulate melatonin production, including drugs, environment, developmental and stress, it may be possible to regulate the production of this compound, and to predict when production would be enhanced.

Our studies on circadian rhythms in animals may have direct influence on the modern day problem of "jet lag". If we can alter the rate at which new circadian patterns are established in rats and hamsters after a shift in lighting schedules, it seems possible that this might be applied to humans to prevent "jet lag".

Proposed Course of Project: (1) Studies on the neural structures involved in the regulation of the pineal melatonin rhythm in the rhesus monkey will continue. (2) Experiments in the rhesus monkey will be performed of a pharmacological nature, designed to shed light on the transmitters involved in regulating pineal melatonin rhythm. These studies will include analysis of animals in which the superior cervical ganglia, which probably innervates the pineal gland, has been removed. This will allow the evaluation of the effects of administration of drugs on the pineal gland in this situation in which neural elements innervating the pineal gland are absent.

Publications:

1. Reppert, S.M., Perlow, M.J., Tamarkin, L. and Klein, D.C.: A diurnal melatonin rhythm in primate cerebrospinal fluid. Endocrinology 104, 295-306, 1979.

2. Klein, D.C.: Circadian rhythms in the pineal gland. In D. Krieger, ed.: Endocrine Rhythms; Comprehensive Endocrinology 1, Raven Press, New York, 1979, pp. 203-224.

3. Reppert, S., Perlow, M., and Klein, D.C.: CSF Melatonin. In J.H. Wood, ed.: Neurobiology of Cerebrospinal Fluid, Plenum Press, New York, (in press).

4. Perlow, M.J., Reppert, S.M., Tamarkin, L., Wyatt, R., and Klein, D.C.: Photic regulation of melatonin: Man and monkey are not the same. Brain Research (in press).

5. Reppert, S. and Klein, D.C.: The pineal gland: basic and clinical aspects. In M. Motta, Ed: Endocrine Functions of the Brain; Comprehensive Endocrinology IV, Raven Press, New York (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00095-09 LDN

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)
Regulation of Neuroendocrine Metabolism: Transsynaptic Mechanisms in the Pineal Gland

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D.C. Klein	Physiologist	LDN	NICHD
OTHER:	J.L. Weller	Biologist	LDN	NICHD
	L. Tamarkin	Guest Worker	LDN	NICHD
	D. Auerbach	Clinical Associate	NPMB	NICHD
	G.H.T. Wheler	Visiting Fellow	LDN	NICHD

COOPERATING UNITS (if any)

G. Aurbach, MDB, NIAMMD.

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Neuroendocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project focuses on the transsynaptic mechanisms which are important in regulating metabolism of a neuroendocrine tissue, the pineal gland. The specific topics of interest include the interaction during differentiation of the pineal gland and innervating structures, the role of nerves in both releasing and taking up transmitters, the interaction of transmitters in transsynaptic regulation and the characterization of postsynaptic adrenergic receptors.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To describe the transsynaptic mechanisms through which neuronal activity regulates biochemical activity in the pineal gland.

Methods Employed: (1) Cultures of pineal tissue are used to study the molecular mechanisms through which neuronal activity regulates biochemical activity. These preparations are treated with neurotransmitters, drugs, and hormones to determine their effects on pineal cell metabolism. (2) To study development, pineal glands are removed at various ages and the activity of metabolites and enzymes are measured. The animals are also treated with drugs known to block specific neural processes. (3) Beta-adrenergic receptors are characterized using a radioligand binding assay.

Major Findings: (1) Studies on the role of taurine in pineal indoleamine metabolism have provided several interesting new findings. First, it has been discovered that taurine is rapidly released from pineal cells treated with norepinephrine. Norepinephrine acts to release taurine through a beta-adrenergic mechanism. Similar compounds can also have this effect although all appear to act via interaction with the beta-adrenergic receptor. The effects of norepinephrine on the release of taurine are mediated by cyclic AMP, as it has been found that norepinephrine elevates pineal cyclic AMP and that taurine can be released by derivatives of cyclic AMP. Thus, it would appear that one of the most rapid effects of norepinephrine on the pineal cell is to cause rapid release of taurine.

Previous studies had shown that taurine can have distinct effects on the pineal cell, and that these effects are mediated by a beta-adrenergic receptor. Thus, it appears possible that the release of taurine into the extracellular space might have some transsynaptic functional role. The finding that taurine can interact with the beta receptor, which has been confirmed using beta-adrenergic receptor analysis techniques, raises the possibility that taurine might also interact with norepinephrine-binding proteins, located in pretranssynaptic structures involved in either uptake of norepinephrine into nerve endings or the regulation of this uptake process.

(2) The beta-adrenergic receptor on pineal cells has been characterized using a new radioligand. It appears that there are about 10,000 receptors on each pineal cell and that these receptors are functionally associated with the enzyme adenylate cyclase. In addition, it appears that there is a rapid change in the affinity of these receptors for naturally occurring catecholamines and that this results in a rapid desensitization of the receptors.

Significance to Biomedical Research and the Program of the Institute: The pineal gland represents one of the most attractive models of adrenergic control mechanisms presently available. Our understanding of the transsynaptic mechanisms involved in regulating this tissue will lead to a fuller understanding of neural functions in humans and especially in the development of neural control mechanisms. It may be possible, with an increased understanding

of the specific structures and proteins involved in neural transmission and neural biochemical transduction, to identify a set of specific proteins required for normal adrenergic functioning. With these markers in hand it may be possible to evaluate neural disease against these standards and thus to fully diagnose these neural diseases on a molecular basis. This should provide a thorough understanding of the nature of alterations in neural development and mental retardation and also help provide a means of designing strategies to solve problems associated with these issues.

Proposed Course of Project: The effect of taurine on transsynaptic control of adrenergic function will be studied. The mechanism underlying receptor desensitization will be analyzed.

Publications:

1. Buda, M. and Klein, D.C.: Dispersed pinealocytes in culture. Endocrinology 103, 1483-1493, 1979.

2. Wheler, G.H.T., Weller, J.L., and Klein, D.C.: Taurine: Stimulation of pineal N-acetyltransferase activity and melatonin production via a beta adrenergic mechanism. Brain Research 166, 65-74, 1979.

3. Wheler, G.H.T. and Klein, D.C.: Function and regulation of taurine in the pineal gland. in: S.I. Baskin, J.J. Kocsis, and S.W. Shaffer, eds.: Actions of Taurine on Excitable Tissues, Spectrum Press, New York (in press).

4. Wheler, G.H.T. and Klein, D.C.: Norepinephrine stimulates the release of [^{14}C]taurine from the pineal gland. Brain Research (in press).

5. Wheler, G.H.T. and Klein, D.C.: Cyclic AMP induced release of [^{14}C]-taurine from pinealocytes. Biochemical Biophysical Research Communications (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00096-09 LDN

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)
Regulation of Neuroendocrine Metabolism: Intracellular Mechanisms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D.C. Klein	Physiologist	LDN	NICHD
OTHER:	A. Namboodiri	Visiting Fellow	LDN	NICHD
	J.L. Weller	Biologist	LDN	NICHD

COOPERATING UNITS (if any)
K.L. KIrk, LC, NIAMMD; J. Pierce, OD, NHLI; L. Grouse, C. Letendre, LDN, NICHD.

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Neuroendocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Investigations have centered on understanding on a molecular level the intercellular regulation of metabolism in a neuroendocrine organ, the pineal gland. The topics of specific interest include the factors regulating cyclic AMP production, the function of cyclic AMP, the control of synthesis of pineal N-acetyltransferase activity, the control of activation and inactivation of this enzyme, and the role of mRNA synthesis in the regulation of the activity of N-acetyltransferase activity.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To describe the intracellular mechanisms through which neuronal activity regulates biochemical activity in the pineal gland.

Methods Employed: (1) The experimental systems used to study the intracellular mechanisms through which neuronal activity regulates biochemical activity are cultures of pineal glands and broken cell preparations. The intact preparations are treated with neurotransmitters, drugs, and hormones to determine their effects on pineal cell metabolism. Other measures of cell function are studied, including the generation of cyclic nucleotides, the production of new protein, the production of new RNA, the activity of specific enzymes, and the production of metabolic products, including several tryptophan derivatives. (2) Broken cell preparations are used to study the regulation of pineal N-acetyltransferase activity. In addition this enzyme is purified.

Major Findings: (1) Important new progress has been made in purifying pineal N-acetyltransferase from the sheep; using the methods developed for the sheep, methods have also been developed for the isolation of pineal N-acetyltransferase from the rat pineal gland. It has been possible to achieve over a 200-fold purification using methods which are based on hydro phobic and affinity chromatography. (2) The activity of pineal acetyl CoA hydrolase has been found to be activated by cystamine. This activation is based on disulfide exchange. (3) Perhaps the most significant new discoveries made in this laboratory within the last year have to do with understanding the regulation of pineal N-acetyltransferase. As with pineal acetyl CoA hydrolase, it appears that the activity of pineal N-acetyltransferase may be regulated through a mechanism described as disulfide exchange. A number of in vivo and in vitro experiments strongly support the physiological importance of this mechanism in regulating pineal N-acetyltransferase. (4) A large number of experiments have now clearly established that the induction of pineal N-acetyltransferase requires the synthesis of new mRNA. (5) The findings of a common mechanism regulating both pineal N-acetyltransferase and pineal acetyl CoA hydrolase has raised the possibility that a reciprocal relationship in activity of these two enzymes may persist in the cell. This relationship is striking in that it appears to work in a coordinated manner to produce precise control over the production of melatonin. On one hand the disulfide exchange would result in a decrease in the activity of N-acetyltransferase. This decrease in N-acetyltransferase would be enhanced by the activation of acetyl CoA hydrolase because it would destroy acetyl CoA. Acetyl CoA has two functions in pineal indoleamine metabolism. First it is the most potent known stabilizer of the unusually labile pineal N-acetyltransferase. Second, it is a substrate required in the synthesis of melatonin. Thus, the activation of hydrolase would favor a rapid decrease in melatonin production and work in a manner that would be complimentary to turn off melatonin production. The rapid turn off of melatonin production is a well established physiological phenomen.

Significance to Biomedical Research and the Program of the Institute: The pineal gland offers perhaps the most attractive model system for the study of the neural control of gene expression. Although other neural systems are available, none is capable of exhibiting such large and rapid changes in the expression of single gene as is the pineal gland. In addition, with the knowledge and confidence that the increase in pineal N-acetyltransferase depends upon the synthesis of new messenger RNA, it is now appropriate to determine whether or not the neural regulation of this gene, which must be considered to be a high information-low abundance gene, is similar to gene regulation seen in other tissue, as studied through the analysis of major gene products. Our understanding of the factors which control rapid changes in enzyme activity as a function of neural stimulation will be of great importance in understanding the neural control of metabolism in general. It is highly possible that transmitters control many enzymes in a manner similar to that in which norepinephrine controls N-acetyltransferase. Knowing what we know now about the regulation of pineal N-acetyltransferase, we may be able to use these lessons to study and analyze the regulation of enzyme activity in other neurally-controlled tissues, including neurons from the central nervous system.

Proposed Course of Project: (1) The isolation and characterization of pineal N-acetyltranasferase. (2) The generation of antibodies against pineal N-acetyltransferase. (3) The isolation of the mRNA required for the stimulation of pineal N-acetyltransferase. (4) The isolation of newly synthesised molecules of N-acetyltransferase, to determine their rate of turnover. (5) The further investigation of the role of disulfides in the regulation of pineal N-acetyltransferase, and the eventual isolation of an endogenous disulfide involved in the regulation of the activity of this enzyme.

Publications:

1. Wheler, G.H.T., Weller, J.L., and Klein, D.C.: Taurine: Stimulation of pineal N-acetyltransferase activity and melatonin production via a beta adrenergic mechanism. Brain Research 166, 65-74, 1979.

2. Namboodiri, M.A.A., Namboodiri, M.A., Weller, J.L., and Klein, D.C.: A Comparative study of pineal N-acetyltransferase and acetyl CoA hydrolase activities. Journal of Neurochemistry 32, 31-34, 1979.

3. Namboodiri, M.A.A., and Klein, D.C.: Use of Charcoal Adsorption for the microassay of Acetyl-CoA Hydrolase. Analytical Biochemistry 93, 244-247, 1979.

4. Namboodiri, M.A.A., Nakai, C., and Klein, D.C.: Effects of selected treatments on the stability and activity of pineal serotonin N-acetyltransferase. Journal of Neurochemistry (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00097-09 LDN

PERIOD COVERED
October 1, 1978 through September 31, 1979

TITLE OF PROJECT (80 characters or less)
Regulation of Neuroendocrine Metabolism: Melatonin Physiology (rat, hamster, rhesus monkey)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D.C. Klein	Physiologist	LDN	NICHD
OTHER:	S. Reppert	Clinical Associate	NPMB	NICHD
	L. Tamarkin	Guest Worker	LDN	NICHD

COOPERATING UNITS (if any)
M. Perlow, M. Mishkind, L. Uaigerleiter, NIMH

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Neuroendocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Efforts are directed at fully describing the physiology of a putative neuroendocrine hormone, melatonin. Current interest is centered on describing the amount of this compound present in body fluids throughout life.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To study the factors regulating the amount of circulating melatonin, a putative pineal hormone during life.

Methods Employed: (1) Melatonin metabolism, fate, and distribution is studied using [^{3}H]melatonin. Thin layer chromatography is used to isolate [^{3}H]melatonin and its metabolites. (2) Melatonin is measured by radioimmunoassay.

Major Findings: (1) In studies involving pregnant rhesus monkeys and pregnant ewe it was discovered that radioactive melatonin is rapidly transfered across the placenta. In addition, using low concentrations of melatonin it was also found that the dynamics of transfer were such a nature as to make it highly probable that the rhythm in melatonin in the mother would be transmitted to the fetus quite rapidly. In associated studies, it was also found that the fetal pineal gland of the monkey and sheep has the ability to make melatonin. Thus, it would seem highly probable that thoughout the development of the fetus that melatonin from either the mother or from the fetus itself would be available. (2) Studies aimed at determining whether or not there are dietary controls over the amount of melatonin made have indicated that high concentrations of tryptophan can alter the amount of melatonin in the pineal gland.

Significance to Biomedical Research and the Program of the Institute: A growing body of knowledge points to the pineal gland as the source of a compound that inhibits reproduction, and that this compound is melatonin. An increase in our understanding of how melatonin production is regulated, when production is first regulated, when production is initiated, the relative importance of melatonin from mother's milk, the fate of melatonin, and the biological effects of melatonin analogs and metabolites is in accord with the Institute's mission to study reproduction.

Proposed Course of the Project: (1) To accurately determine the amount of melatonin that is transferred to the suckling by milk, and the precise amounts of melatonin in the circulation of the suckling. (2) To determine whether or not normal flucuations in dietary tryptophan alter melatonin production.

Publications:

1. Reppert, S., Chez, R.A., Anderson, A., and Klein, D.C.: Maternal-fetal transfer of melatonin in the non-human primate. Pediatric Research (in press).

2. Tamarkin, L., Reppert, S.M., and Klein, D.C.: Regulation of pineal melatonin in the Syrian Hamster. Endocrinology 104, 385-391, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00700-02 LDN

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Cell interactions in synaptogenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C.N. Christian	Staff Fellow	LDN NICHD
	M.P. Daniels	Biologist	LBG NHLBI
OTHER:	P.G. Nelson	Chief, LDN, NICHD	LDN NICHD
	H.C. Bauer	Guest Worker	LDN NICHD
	J. Prives	Guest Worker	LDN NICHD
	L. Jacques	Student Scientist	LBG NHLBI
	P. Pudimat	Bio. Lab. Tech.	LDN NICHD
	M. Jackson	Guest Worker	LB NINCDS
	D. Axelrod	Bioph. Res. Div.	Univ. of Michigan

COOPERATING UNITS (if any)
Laboratory of Biochemical Genetics, NHLBI, Neurobiology Department, The Weizmann Institute of Science; Laboratory of Biophysics, NINCDS; Biophysics Research Division, University of Michigan

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Neurobiology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	2.5	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is the investigation of the cellular and molecular interactions during synaptogenesis. It has focused on a factor found in the medium conditioned by cholinergic neuronal cells which aggregates the acetylcholine receptors on muscle cells. The factor was also found in extracts of embryonic brain but was not detected in adult brain, and hence is developmentally regulated. The factor has been partially purified and characterized.

Medium conditioned by cholinergic neuronal cells was also discovered to stabilize acetylcholine receptors by apparently promoting their attachment to the myotube cytoskeleton. Conditioned medium also decreased the lateral mobility of myotube acetylcholine receptors and modified the distribution of the single channel kinetics of the acetylcholine receptor.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: We are exploring the problem of synaptic specificity by a molecular analysis of some of the processes which occur when pre- and post-synaptic cells interact. The processes we are studying include the juxtaposition of pre-synaptic release sites with post-synaptic receptors, the stabilization of the post-synaptic receptor, and the change in single channel kinetics of post-synaptic receptors.

Methods Employed: In our studies, both pre- and post-synaptic cells have been grown in vitro by tissue culture techniques. Both continuous cell lines and primary neuronal and muscle cells have been used.

Synaptogenesis and the distribution of acetylcholine sensitivity on cultured myotubes have been assayed electrophysiologically by intracellular recording methods.

The aggregation of acetylcholine receptors on myotubes have been assayed electrophysiologically by intracellular recording methods.

The aggregation of acetylcholine receptors on myotubes has been determined by the use of peroxidase or fluorescent probes conjugated to -bungarotoxin, a specific ligand for the acetylcholine receptor. The ultrastructure of acetylcholine receptor aggregates labelled by the peroxidase method has been investigated with an electron microscope.

Standard biochemical methods are being employed to purify, isolate and characterize the factor which causes acetylcholine receptor aggregation.

The lateral mobility of acetylcholine receptors has been asseyed by the Fluorescence Recovery after Photobleaching.

The kinetics of the acetylcholine receptor channel have been studied by noise analysis and direct recording of single channels with patch electrodes.

Major Findings: A factor released from the neuronal cells NG108-15 has been partially purified and characterized, employing the bioassay of its aggregation of muscle acetylcholine receptors. The molecular weight of the factor was determiend by molecular seiving, and its isoelectric point found by preparative isoelectric focusing. A factor with similar physical properties and also causing the aggregation of muscle acetylcholine receptors was discovered in extracts from embryonic rat brain but was not detected in adult brain.

It was discovered that glial cells and certain other non-neuronal cells produce a factor which decreases the number of acetylcholine receptor aggregates on myotubes. A similar aggregation inhibition

activity was separated from aggregation activity in medium conditioned by NG108-15 cells during purification. The localization of muscle acetylcholine receptors at the neuromuscular junction may be the net effoect of a neuronal aggregating activity and the aggregation inhibitory effects of other cell types.

When concentrated more than 20 fold, the factor found in NG108-15 cell conditioned medium produced a maximal effect on acetylcholine receptor aggregation within 2 hours. The aggregation is produced by the laterial movement of acetylcholine receptors in the muscle cell membrane. The effect was temperature dependent, and was largely blocked by colchicine, glucosamine, and tertiary amines.

The aggregation of the acetylcholine receptors by the neuronal factor is followed by the stabilization of the aggregated receptor, possibly by attachment to the myotube cytoskeleton. The rate of extraction of muscle cell acetylcholine receptors by a non-ionic detergent was determined by quantitative fluorescence microscopy. Receptors found in aggregates were extracted more slowly than diffusely distributed receptors. The rate of detergent extraction of receptors from muscle cultures was slowed by the same concentrations of NG108-15 cell conditioned medium that produced receptor aggregation. The onset of the conditioned medium stabilization of acetylcholine receptors followed the onset of receptor aggregation.

The mechanism of the laterial motion of acetylcholine receptors induced by NH108-15 cell conditioned medium was investigated using fluorescence photobleaching. Conditioned medium decreased the lateral mobility of diffusely distributed myotube acetylcholine receptors.

Taken together, these findings suggest that the neuronal factor promotes the attachment of acetylcholine receptors to muscle cell cytoskeletal structures, which actively pull together the receptors to form a patch.

Proposed Course: The biochemical purification and isolation of the aggregation factor will allow us to determine the causal connection between the various effects observed when muscle cells are treated with neuronal conditioned medium. The sequence of molecular events by which the neuronal aggregation factor interacts with muscle cell acetylcholine receptors will be explored and extended to synaptogenesis and intercellular recognition.

1. Christian, C.N., Daniels, M.P., Sugiyama, H., Vogel, Z., and P.G. Nelson. A factor from neurons increases the number of acetylcholine receptor aggregates on cultured muscle cells. Proc. Nat. Acad. Sci., 75:4011-4015, 1978.

2. Christian, C.N., Nelaon, P.G. Synapse formation of continuous cell lines. Pontifical Academy Sciences Meeting, Rome, Italy., p. 161-180., 1979. In press.

NICHD ANNUAL REPORT
October 1, 1978 through September 30, 1979

Laboratory of Molecular Genetics

The Laboratory of Molecular Genetics conducts research directed towards understanding the molecular processes involved in the transmission of genetic information and in its regulated expression during growth and differentiation. A variety of model systems are under investigation, ranging from bacterial and animal viruses to complex vertebrate regulatory responses involving the synthesis of specific antibodies and the development of specific organ systems. Taken together, the laboratory joins active workers in the areas of genetics, biochemistry, electron microscopy, immunology, virology and cellular biology to bring their skills to bear on problems of mutal interest. At present the laboratory is comprised of nine research groups distributed among six sections.

I. Section on Gene Organization and Control

A. Studies have been directed towards understanding the regulated expression and organization of two sets of mammalian genes, those involved in globin and immunoglobulin production. An approach involving the use of recombinant DNA technology has been developed that permits the cloning of virtually any segment of the mammalian genome for which there is a hybridization probe. Using this approach, a large number of chromosomal genes from both systems have been cloned. The entire structures of the mouse β^{maj}-, β^{min}- and α-globin genes have been determined. A comparison of their sequences suggests that insertion-deletion mechanisms in addition to single base alterations were principally responsible for their rapid evolutionary drift. Further comparison permits identification of putative transcriptional regulatory signals and processing sites. A functional test for such signals has been devised using these chromosomal genes cloned in the mammalian virus SV40. Infection of cultured cells with the hybrid virus results in considerable globin production indicating that the system will be quite useful. Cloning and sequence studies in the immunoglobulin system have clarified the basis of antibody diversity as depending upon a large array of germline variable region genes (V) that recombine with one of five joining region segments (J) that encode a 13 aminoacid sequence at the variable/constant region border. The combination of V's and J's generates considerable diversity both from the simple combinational property as well as from an alteration of the frame of recombination. In addition, an inverted repeat sequence that might be important for V/J recombination has been noted adjacent to the recombination site of all V and J region genes. (Dr. Philip Leder - Principal Investigator)

B. Studies have been directed towards understanding the regulatory pathways controlling the antibody response at the cellular level. The integrated roles of regulatory T-lymphocytes, macrophages and responder B-lymphocytes are being delineated in vitro using cells obtained from athymic (nu/nu) mice and normal (+/nu or +/+) NFR/N mice. A streptococcal exotoxin (SPE) has been shown to profoundly affect antibody responses apparently by cancelling the function of suppressor T-lymphocytes. The functions of macrophages and B-lymphocytes are not directly altered. Two different populations of T-lymphocytes (Pools 1 and 2) have been separated on solid-phase (nylon-wool) columns. The SPE effect is observed only when pool 2 is treated with SPE and recombined with the other non-treated cells of the antibody forming system. This thus localizes the suppressor cell population to pool 2. All cells appear to bind SPE as detected by immunofluorescence techniques, but they do so with different site and activation patterns. Macrophages appear to engulf SPE and are not obviously inhibited in their function. A small population of B-lymphocytes exhibit a patchy localization of adsorbed SPE on their surface which they slowly or rapidly polarize or "cap" and shed, with some dependence on the molecular size of the SPE. The receptor for SPE on B-lymphocytes may be surface immunoglobulin. T-lymphocytes bind SPE in a diffuse pattern and appear incapable of capping and shedding the SPE. Instead T-lymphocytes appear to slowly relocalize the diffusely bound SPE internally, by a mechanism not preceded by capping. Experiments are now directed towards discovering differences in the fate of SPE within the different types of T-lymphocytes as they relate to altered cell function. Evidence indicating SPE induced transitions among T-lymphocytes is sought. In this respect, a shift in the activities of Pool 1 and Pool 2 T-cells fractionated from SPE pretreated donor mice was observed. Under this condition the SPE effect was generated by both populations of T-cells (Pool 1 and Pool 2). Thus it appears that SPE, a natural bacterial probe may derive T-cells along functional pathways. Hybridomas between TNP-immune B-cells and the P3X63Ag8 subline of MOPC-21 which secrete monoclonal anti-TNP antibodies are being established. Concurrently T-cell hybridomas with the activities of Pool 1 and Pool 2 cells are sought.
(Dr. Edgar Hanna - Principal Investigator)

C. Studies have been directed towards understanding the molecular events involved in the assembly of RNA tumor viruses. A major question concerns the mechanism by which virions encapsidate a selected population of host tRNA molecules. Although a virus-specified function appears to be involved, we have shown that the viral RNA genome is not a critical determinant in tRNA selection. Using a genetic approach, our focus has shifted to the possible role of viral RNA binding proteins, in particular the reverse transcriptase enzyme which is known to interact with the tRNA primer for RNA-directed DNA synthesis. Two non-conditional murine leukemia virus mutants have been used: one (M13) is missing the reverse transcriptase; and the other (clone 23), which we have helped to characterize, contains only 2-5% of normal wild type activity. This low level of activity appears to be associated with a molecule which is 20% smaller than the normal enzyme. Using a two-dimensional gel system to analyze viral tRNA, we have found that compared to wild type, there is a significant decrease in the amount of primer tRNA present in both of the mutants. Preliminary results suggest that other viral tRNAs are not greatly affected by a mutation in the reverse transcriptase gene. Possible effects of different viral proteins on tRNA assembly will be investigated with other virus mutants. In addition, mapping studies on the clone 23 mutant will be carried out. Work

has also continued on the role of the viral RNA genome in other aspects of virus replication. Studies with virus particles lacking genomic RNA as well as thermal inactivation experiments correlating loss of intact genomic RNA with biological activity indicate that the viral RNA genome is required for abrogation of the Fv-1 host restriction system which regulates murine leukemia virus infection. (Dr. Judith Levin - Principal Investigator)

II. Section on Cellular Differentiation

Studies have been directed towards understanding the molecular events pertaining to gene expression and protein synthesis in the vertebrate lens during development and disease (cataract). Crystallin synthesis has been investigated in the developing chick lens and in two hereditary cataracts in mice. Analysis of the chick lens has shown that δ-crystallin (the principal protein of the embryonic lens) is composed of at least two different polypeptides (50,000 and 48,000 daltons). The ratio of synthesis of the δ-crystallin polypeptides is dependent, in vivo and in vitro, upon the anion and cation concentrations. Evidence suggests that the larger polypeptide is the primary translational product, and that under physiological ionic conditions 75% of these are cleaved at the C-terminal end during translation to produce the smaller polypeptide. δ-Crystallin cDNA has been cloned in the bacterial plasmid pBR322, and two EcoR1 genomic fragments of the δ-crystallin gene have been cloned in λ Charon 4A. The two genomic clones have been oriented with respect to one another. These two δ-crystallin DNA fragments have 12 introns. Four other EcoR1 genomic fragments containing δ-crystallin sequences have been isolated. Comparative studies have demonstrated the conservative nature of the structure of δ-crystallin, and investigations have been initiated comparing the δ-crystallin genes in chickens and ducks. Chicken β-crystallin polypeptides have been compared and experiments to isolate their mRNAs have been started. Finally, studies on the Nakano and Philly hereditary mouse cataracts have supported the idea, based on the δ-crystallin experiments, that the intracellular concentration of electrolytes differentially affects the synthesis and degradation of crystallins.
(Dr. Joram Piatigorsky - Principal Investigator)

III. Section on Molecular Structure

Studies have been directed towards understanding the structure of animal virus genomes and the way viruses gain control of the host cell's molecular processes. Research has been concentrated in three major areas: poliovirus replication, early events in adenovirus replication, and methodology. Poliovirus genomes have been mapped electron microscopically, biochemically and genetically. A map of the positions of 40 RNAse T1-resistant oligonucleotides has been correlated with the positions of secondary structure features detected by electron microscopy and ribonuclease III digestion. Deletions in newly isolated defective interfering particles have been mapped by the absence of oligonucleotides, by deficiencies in proteins synthesized from the 5' position of the genome, and by EM heteroduplex analysis. Translational, initiation-specifi ribosome binding sites have been positioned at three major sites at both ends and internally in the genomes. Other sites may be involved. Adenovirus interactions with the host cell have been analyzed by careful subcellular localization of the

early proteins and analysis of their biosynthesis, and post-translational modification. The precursor of a 19K (MW_{app}) protein is a 15K non-glycosylated protein as proven by peptide mapping. Peptide mapping studies on deletion mutants and proteins translated *in vitro* have correctly reassigned the map position for the 15/19 gene from early region IV (assigned by work of others) to early region III of the genome. All of the adeno virus early proteins are intimately associated with the cytoskeleton of the cell. Electron microscopic techniques have been extended through the installation of the biotin-avidin-polyvinyl sphere labelling technique Computerized reading of electron micrographs and mapping of simple molecules have been demonstrated. Refinements to the programs have been undertaken to allow more complex molecules to be mapped. Nucleic acid sequence analyses have been greatly improved by development of a practical, graphic method for revealing patterns in large sequences. A glance at the esthetically pleasing patterns clearly reveals relatedness between pairs of sequences, or within sequences, and can predict secondary structures. Programs to calculate stabilities of secondary structures have been obtained and are being optimized. Collaboration applying techniques and principles continue to be a major activity.
(Dr. Jacob V. Maizel, Jr. - Principal Investigator)

IV. Section on Animal Viruses

Studies have been directed towards understanding the transcription and processing of mRNA's from the human adenovirus. Spliced forms of early adenovirus messenger RNA have been detected, the structures of all viral RNA species found in the nucleus and cytoplasm of cells harvested before the onset of viral DNA replication have now been determined. The results of this survey allow an insight into the intricate controls of early virus gene expression. In collaboration with colleagues of the Salk Institute in San Diego and at NIAID, the structure and expression of SV40 sequences present in the nondefective hybrid virus Ad2+ND_4 was also examined. An interesting and most unexpected outcome of these studies is the fact that monkey and human cells differ in functions (possibly splicing enzymes) that mediate the expression of these SV40 sequences. Finally, the techniques of microinjection of nucleic acid into mammalian cells have been established and refined with the aim of analyzing details of the process of eukaryotic RNA processing and expression.
(Dr. Heiner Westphal - Principal Investigator)

V. Section on Molecular Regulation

A. Studies have been directed toward understanding how cells coordinate the expression of their genome during growth and in particular how the regulatory nucleotide ppGpp participates in this control. Evidence indicates that ppGpp probably controls initiation of rRNA transcription, most tRNA transcription, ribosomal protein gene transcription and about half of the remaining structural genes in *E. coli*. We have isolated and partially characterized the first mutant incapable of ppGpp synthesis to demonstrate that the nucleotide is dispensable, except for regulatory functions much the same as cAMP. Studies on initiation of rRNA transcription from cloned DNA fragments of the *rrn*B cistron have revealed two tandem promoters; this

finding has been generalized by others to include five of the seven other ribosomal cistrons. The rrnB cistron has been sequenced by the nicked translation method and is generally identical to the rrnA cistron with the two sequences diverging at 369 base pairs upstream from the mature 5' end of 16S rRNA (80 base pairs upstream from the earliest promoter). Analysis of the structure function relationships of this complicated promoter seems dependent upon a simple in vivo assay of promoter function under conditions where copy number can be tightly controlled. The rrnB promoter region has been fused to the middle of the galactose epimerase gene and shown that galK and T genes are expressed with the same growth rate control and stringent control as ribosomal cistrons. This system should allow for positive and negative selection pressures for mutant isolation as well as quantitative assays for function of engineered promoter alterations. Antibody to ppGpp has been prepared allowing a sensitive radioimmunoassay for the 3' pyrophosphoryl residue on guanosine. (Dr. Michael Cashel - Principal Investigator)

B. Studies have been directed toward understanding the role of certain ribonucleases in processing RNA. Studies of the processing of ribosomal RNA from chick embryos by RNase DII, an enzyme first isolated in this laboratory from chick embryos, has centered on the primary and secondary structure of the sequences involved in processing. Restriction enzyme analysis and sequence determination suggest that at least one of the cleavage sites is formed by the 3' terminus of 5.8S rRNA forming a stable duplex with the 5' terminus of 28S rRNA and cleavage occurs at--or near--the mature ends. In a separate series of experiments on RNaseH from E. coli, results have been obtained demonstrating that the enzyme acts as a dimer--RNaseH is involved in generating primers for DNA replication. This model will be tested shortly using the information developed on the location on the E. coli chromosome of a mutant that has lower levels of RNaseH. (Dr. Robert Crouch - Principal Investigator)

VI. Section on Microbial Genetics

Studies have been directed towards understanding the mechanism by which a virus, the bacteriophage λ, inserts its chromosome into and excises it from the chromosome of its host. Both insertion and excision are examples of a general process called site specific recombination. Mutations have been isolated and characterized in three genes, two phage and one host, that encode proteins required for λ site-specific recombination. The characterization of mutations in the gene encoding the phage int protein suggests that a different form of this protein is needed for insertion than for excision. Two cases have been found in which a cycle of insertion and excision changes the structure of the attachment sites, the DNA substrates for the two reactions. One likely explanation of this finding, which is being tested by nucleotide sequence analysis, is that a different internucleotide bond was cut during excision than during insertion. Unlike the int gene, the other phage and host genes involved in site specific recombination are not absolutely required for both insertion and excision and may therefore be considered as accessory factors. A phage mutant has been isolated that shows a reduced requirement for these accessory factors. This mutant also has the novel property of stimulating recombination between attachment sites that normally recombine only at very low frequency. Characterization of this mutant promises to shed light on the roles of the accessory factors. (Dr. Robert Weisberg - Principal Investigator)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00067-11 LMG

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)
Integrative Control of Macromolecular Synthesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael Cashel	Research Medical Officer	LMG NICHD
OTHERS:	Yoshimi Ota	Visiting Fellow	LMG NICHD
	James Sylvester	Staff Fellow	LMG NICHD

COOPERATING UNITS (if any)
Dr. A.G. Atherly, Iowa State University; Dr. P. Nelson, LMG, NICHD; Dr. A. Kikuchi, LMG, NICHD

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Molecular Regulation

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project continues is directed towards understanding how a cell coordinates the expression of its genetic repertoire during both normal growth and unbalanced nutritional deficiencies. Previous project work has described the discovery, characterization and regulatory attributes of a regulatory nucleotide (ppGpp) that is thought to participate in the regulation of about half of the genes of E. coli.

Currently, this regulatory role of ppGpp is verified by the physiological and genetic characterization of a new mutant defective in ppGpp production. In addition, we have constructed an operon fusion such that the ribosomal promoter from the rrnB operon directs transcription of the galK and galT genes in the galactose operon. The existence of this fusion strain opens new approaches towards understanding how ppGpp can regulate gene expression.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The continued aim of this project is to understand in molecular terms how cellular activities are coordinated. This regulation is a prerequisite to normal growth but is most prominently displayed during cellular responses to change in growth conditions. The regulatory system studied in detail here is mediated by ppGpp, a nucleotide effector formed on ribosomes when messenger RNA codons bind to uncharged cognate tRNA. Classically, mutants unable to respond to uncharged tRNA by synthesizing ppGpp have defined this regulatory system but have nevertheless accumulated ppGpp during energy source deprivation. Previous annual reports have considered ppGpp specified regulatory consequences on cellular intermediary metabolism, transport and cellular physiology. Here we continue to dissect out characteristics of ppGpp production as well as the way in which ppGpp regulates initiation of gene expression.

Principal Findings:

1. Biosynthesis of ppGpp. Last year's annual report briefly described the isolation of a new mutation called relS that results in a seemingly absolute deficiency in ppGpp accumulation in a host that lacks the ability to synthesize large amounts of ppGpp. During the past year this mutation was more accurately localized by conjugal transmission gradients and F' mapping and the regulatory effects of the mutant allele examined in more detail.

The relS mutation is localized to the 12-14 min region when gal$^+$ recombinants of a Hfr KL16 mating are screened for lac recombination and ppGpp production. The relS allele is complemented by F'254 but not F'42, F'113 or F'152. These observations localize the relS mutation to the 12-14 min region also. Since merodiploids of F'254 lose the ppGpp accumulation phenotype when cured of the episome, it is evident that relS is recessive to the relS$^+$ allele. This suggests that relS does not encode an enzyme capable of extremely rapid ppGpp decay.

Physiological studies have indicated that the relS mutant strain is able to grow (albeit slowly) on minimal media. When the medium is supplemented with amino acids, there is virtually no difference in growth rate between a relS mutant and its relS$^+$ parent. The only other difficulty peculiar to the mutant so far detected is an almost total inability to survive withdrawal of amino acids from the medium. This is manifested by extremely long lag times before growth resumption after amino acid downshift. Conversely when amino acids are added back to otherwise minimal media, growth resumption is as rapid as in the parent strain.

The relS mutation seems to define an enzyme for ppGpp synthesis that coexists with the ribosomal ppGpp synthetic system described earlier. It also provides a means of examination of regulatory effects when ppGpp is

absent and thereby affords a means of learning of new regulators with effects independent of ppGpp.

An antibody prepared against ppGpp in this laboratory has been characterized and used for a highly specific radioimmunoassay in collaboration with Dr. A. Atherly at Iowa State University. This assay should afford a means of rigorously assessing the ppGpp content in eukaryotes.

2. Regulation of Intiation of Gene Expression by ppGpp. Last year's annual report described isolation of an initial section of a gene that is negatively controlled by ppGpp, one ribosomal RNA cistron of the seven such genes in E. coli called rrnB. Our findings that the initial section has two tandem promoters about a hundred bases apart has since been confirmed in other laboratories for four other ribosomal RNA cistrons (rrnA, rrnD, rrnE and rrnX). In cooperation with Dr. P. Nelson, we have sequenced the rrnB promoter region. Since there are multiple copies of the genes encoding the rRNA gene products it is not possible to study mutational changes in one due to high backgrounds from the others. To circumvent this problem, we have fused the rrnB promoter region to the first gene in the galactose operon. This fusion was engineered in lambda phage so that copy number could be controlled. Genetic studies have demonstrated that the phage indeed carries the DNA segments intended and these conclusions have been confirmed in physiological studies. We have shown that galactokinase synthesis in the fusion strain has been rendered constitutive, subject to stringent amino acid control and to growth rate control. These regulatory characteristics are as expected for ribosomal promoters, but not a normal gal operator-promoter region. The existence of intact regulation systems in the fusion phage proves that only the initial segment of the gene is required for its regulation. Positive and negative selective pressures are available for gal gene expression to facilitate isolation of regulatory mutants.

Biochemical studies of initiation of gene transcription by RNA polymerase have allowed the isolation of a stable complex of DNA and RNA polymerase dependent upon the formation of a single phosphodiester band. We have characterized the stability of this DNA-protein-ribonucleotide complex both in terms of parameters affecting its formation and degradation. Release of the oligo ribonucleotide moiety is abortive initiation and can result in formation of many copies of the oligoribonucleotide. Such systems have now been well characterized in this laboratory, with synthetic, simple DNA templates such as d(A-T)n and will be applied to naturally occurring promoters.

Significance to Biomedical Research and the Program of the Institute: Integrative control processes are common to all growing and nutritionally adapting cells. This problem has turned towards a more basic understanding of how gene expression is initiated and regulated which will be essential for understanding primitive, differentially and specialized cellular functions.

Proposed Course of Project:

Genetic and biochemical approaches discussed will be pursued using biochemical verification of mutant behavior to validate the significance of in vitro observations. Ultimately it is hoped regulatory participants will be highly defined and molecular routes of initiation of gene expression will be understood.

Publications:

1. Glaser, G. and Cahsel, M.: In vitro transcripts from the rrnB ribosomal RNA cistron originate from two tandem promoters. Cell 16: 111-121, 1979.

2. Engel, J.A., Sylvester, J. and Cashel, M.: Guanosine 3',5'-bispyrophosphate is a dispensable metabolite. In Koch, G. and Richter, D. (Eds.): Regulation of Macromolecular Synthesis by Low Molecular Weight Mediators. New York, Academic Press, in press.

3. Silverman, R., Atherly, A.G., Glaser, G. and Cashel, M.: In Koch, G. and Richter, D. (Eds.): Regulation of Macromolecular Synthesis by Low Molecular Weight Mediators. New York, Academic Press, in press.

4. Ota, Y., Kikuchi, A. and Cashel, M.: Gene expression of an E. coli ribosomal RNA promoter fused to a galactose structural genes. Proc. Natl. Acad. Sci. U.S.A., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00068-08 LMG

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)
Factors Influencing Genetic Transcription-Initiation and Termination

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert J. Crouch	Research Chemist	LMG NICHD
OTHER:	Patricia E. Nelson	Staff Fellow	LMG NICHD

COOPERATING UNITS (if any)
Margaret Stewart, LMG, NICHD
Dr. Philip Carl, University of Illinois

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Molecular Regulation

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

RNA plays an important role in cellular regulation--either by its presence in active form or by its total absence. A number of proteins have been shown to be involved in regulating RNA synthesis and RNA processing in Escherichia coli--among these are RNA polymerase, rho and RNase III. These proteins are involved in the synthesis or inhibition of synthesis of specific species of RNA and in the processing of RNA. Another enzyme which may be involved in regulation of RNA synthesis is RNase H. The objective of this research project is to determine the method by which several of these enzymes act to regulate RNA synthesis in E. coli and chick embryos.

PHS-6040
(Rev. 10-76)

Major Findings:

Our goal has been to understand the control of synthesis and utilization of RNA transcripts at the enzymological level. Primary transcripts generally are cleaved to smaller products during the course of maturation. We are studying an enzyme from chick embryos that was isolated in this laboratory and implicated in processing ribosomal RNA by studies on isolated nucleoli (the organelle in which synthesis and processing of ribosomal RNA is localized) and by the ability of this ribonuclease, RNase DII, to degrade double-stranded RNA to acid soluble-products--a property shared by one of the E. coli enzymes involved in ribosomal RNA processing, RNase III. Our attention now is focused on the sites of cleavage of ribosomal RNA precursors at the level of the precise nucleotide sequence that is cleaved. We have found one such cleavage site that seems to be generated by two complementary sequences that are separated by approximately 700 nucleotides. Such a cleavage site would fit well with our previous prediction that a double-strand specific RNase would be involved in ribosomal RNA processing.

As part of our understanding of the mechanism of action of RNase III, our studies on the cleavage of polio RNA have continued. Together with M. Stewart, a very complete map of "unique" T1 resistant oligonucleotides has been determined. Such knowledge has been useful in 1) generating specific fragments of RNA, 2) localizing these fragments to different regions on the genome and 3) in collaboration with K. McClain, M. Stewart and J. Maizel demonstrating that these fragmented RNA molecules can be translated into specific protein products in vitro.

Continued research on RNase H, an enzyme that specifically degrades the RNA of DNA-RNA hybrids, has led to some significant observations. First, the mechanism of degradation seems to involve a protein composed of two identical monomers that in a single event cleaves the RNA molecule at two separate sites. This mechanism suggests that RNase H can excise RNA from a DNA-RNA hybrid and yet leave behind oligonucleotides ranging to 9 nucleotides in length--these oligomers could then be used to prime DNA replication. Second, we have mapped the genetic locus responsible for low levels of RNase H in a strain of E. coli isolated by P. Carl. Using Hfr mating, F' mating and P1 transduction, the mutation responsible for the low level of RNase H has been localized at 5.1 min on the genetic map of E. coli. We have selected and characterized a clone from the Carbon-Clarke plasmid collection that will permit us to clone the DNA sequence responsible for the low level of RNase H.

Proposed Course of Project:

We will continue to study the sequences involved in ribosomal RNA processing with particular emphasis on the in vitro cleavage of ribosomal RNA precursors by RNase DII. Utilizing cloned DNA fragments that carry all or only portions of a processing site we will be able to look at the

structural requirements for processing as well as for any proteins that may be specifically involved in regulating the processing event. Studies on RNase H will continue using the RNase H mutant strain and the clones that we will have from both wild type and mutant strains. Such studies should permit us to determine whether the mutant is a result of an alteration in the gene coding for the structure of the RNase H protein or a change in a regulatory protein and permit us to alter the level of RNase H within a cell. Also site specific mutagenesis should give us a cell whose RNase H is completely inactive.

Publication:

1. Grummt, I., Hall, S.H., and Crouch, R.J.: Localisation of an endonuclease specific for double-stranded RNA within the nucleolus and its implication in processing ribosomal transcripts. Eur. J. Biochem., 94, 437-443.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00069-07 LMG

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Molecular Aspects of the Replication of Enveloped Animal RNA Viruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Judith G. Levin Research Biochemist LMG NICHD
OTHERS: None

COOPERATING UNITS (if any)
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Jonathan Seidman, LMG, NICHD
Sisir Chattopadhyay, Pediatric Oncology Branch, NCI

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Gene Organization and Control

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.0	0.3

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goal of this project is to define the molecular mechanisms involved in the replication of enveloped RNA viruses and in particular, to understand the factors which influence the regulation and expression of viral genetic information. The possible role of the viral RNA genome in directing virus assembly as well as certain aspects of viral RNA metabolism are being investigated in a system which uncouples murine leukemia virus RNA synthesis from terminal steps in virus maturation. The virus particles produced under these conditions resemble deletion mutants since they lack the 70S RNA genome and contain predominantly 4S RNA. Another major interest concerns the mechanism by which murine leukemia virus particles encapsidate a selected population of host tRNAs. The possible role of one or more viral proteins in the tRNA selection process is being explored by analyzing the tRNA composition of several nonconditional viral mutants. Recent results indicate that the reverse transcriptase enzyme selects the tRNA primer used for viral RNA-directed DNA synthesis.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To study factors which influence expression of viral genetic information; to define in molecular terms the specificity requirements of the virus assembly process; to understand the virus-host relationship in chronic infection.

Methods Employed: Mouse cells chronically infected with murine leukemia virus are maintained as continuous lines in culture. Leukemia virus production is measured by assay of the extracellular fluids for reverse transcriptase activity in response to synthetic templates. Infectivity is determined by the XC plaque assay. The RNA species present in virions are analyzed by polyacrylamide gel electrophoresis. Conditions have been devised which yield high resolution of a wide range of RNA molecules, from the high molecular weight 70S viral RNA genome to the low molecular weight 8S and 4S RNA species. Viral proteins are analyzed on polyacrylamide slab gels. Electron microscopy is used to follow viral morphogenesis and to delineate the ultrastructural features of the virions.

Major Findings:

1. In earlier work on this project, a system was developed in which synthesis of murine leukemia virus RNA is uncoupled from terminal steps in virus maturation by treating chronically infected cells with actinomycin D. Despite the absence of ongoing viral RNA synthesis, virus particles continue to be assembled for at least 8-12 hours. Most of the virions produced are defective, however, and form a unique and previously undescribed class of RNA tumor virus particles which lack 70S genomic RNA and appear to contain only low molecular weight RNA species. We refer to these defective particles as Act D virions.

2. Studies on viral RNA. RNA tumor viruses contain a selected population of host tRNA molecules. Mechanisms involved in the selection process are being investigated. Our previous results with ActD virions exclude the possibility that the viral RNA genome is a major determinant in this process. Since tRNA selection appears to be a virus-specified function, we now suspect the involvement of one or more viral proteins. Viral tRNA from two nonconditional mutants deficient in the reverse transcriptase enzyme have been analysed using two-dimensional gel electrophoresis. One of these mutants, M13, shows a significant decrease in the amount of proline tRNA, the primer for murine leukemia virus RNA-directed DNA synthesis; other viral tRNAs appear to be unaffected by the mutation. Thus, we conclude that the reverse transcriptase enzyme plays an important role in the selection of the viral primer tRNA.

3. Act D virions have been used as a biologic reagent to probe the role of viral RNA in the Fv-1 host restriction system. Recently, conditions have been established which allow murine leukemia virus particles to infect nonpermissive cells. This phenomenon, termed "abrogation", is

two-hit, i.e., two virus particles are required for each infection; however, only one of the particles must actually be capable of replication. Viral functions provided by the nonreplicating virus have been investigated. Virus particles lacking reverse transcriptase activity (either heated virus or virions with a mutation in reverse transcriptase (see below)) are able to abrogate Fv-1 restriction, indicating that functional reverse transcriptase is not a requirement for abrogation. However, two lines of evidence indicate that intact genomic RNA is required: a) Thermal inactivation of abrogating activity can be correlated with a loss in the amount of intact 35S subunit RNA; and b) Act D virions are unable to abrogate Fv-1 restriction. In addition, since we have already shown that Act D virions are defective only with respect to RNA content and contain normal amounts of all the virion proteins, the results also demonstrate that virus structural proteins are not involved in abrogation.

4. A nonconditional murine leukemia virus mutant, "clone 23", which is deficient in reverse transcriptase has been characterized. The mutant has 70S genomic RNA with a normal 35S RNA subunit structure, contains normal amounts of all of the virion structural proteins, but has only 2-5% of the wild-type level of reverse transcriptase activity. This activity is associated with a molecule which is 20% smaller than the normal enzyme. The mutant has been used in the studies on Fv-1 restriction, cited above.

Significance to Biomedical Research and the Program of the Institute: The advantages of viral systems as models for studying the regulation and expression of genetic information are well recognized. Studies on the RNA tumor viruses are of special interest since these viruses establish a chronic infection in the host cell by integrating viral genetic information into the host chromosome. An understanding of the various steps involved in tumor virus replication and mechanisms for generating defective virions should aid in efforts to limit the oncogenic potential of these agents. Finally, information on the degree to which host material can be assembled into virus particles could have some application in the field of gene therapy, where the use of pseudotype virions has been contemplated as a means of introducing "foreign" nucleic acid into an individual possessing an inherited biochemical defect.

Proposed Course of the Project:

1. In the coming year, efforts will continue to elucidate mechanisms involved in selective packaging of host tRNAs by murine leukemia virus particles. Our approach to this problem will continue to involve analysis of tRNAs from mutant viruses which are missing one or more viral proteins. We hope to examine additional mutants to determine whether proteins other than reverse transcriptase affect tRNA selection.

2. The actinomycin D system will provide an approach to studies on viral RNA metabolism and the regulated expression of viral genetic information. We will take advantage of the observation that virions released from mouse cells infected with helper-independent murine leukemia viruses, such as the AKR virus used in our Act D studies, package a novel class of endogenous viral RNA sequences ("30S RNA"). This 30S RNA is thought to represent the genome of a defective endogenous virus. We are interested in determining whether the intracellular pool of 30S viral RNA and the appearance of 30S RNA in extracellular virions are subject to the same synthetic constraints as the helper-independent virus. In these experiments, which are now in progress, AKR virus-infected cells are incubated in the presence and absence of actinomycin D. Hybridization techniques utilizing specific viral DNA probes are being used to measure intracellular and extracellular sequences of 30S RNA and AKR virus RNA.

3. Further studies on the reverse transcriptase mutant, clone 23, will be carried out. The site of the genetic lesion will be mapped by electron microscopic techniques.

Publications:

1. Levin, J.G. and Seidman, J.G.: Selective packaging of host tRNA's by murine leukemia virus particles does not require genomic RNA. J. Virol. 29: 328-335, 1979.

2. Gerwin, B.I., Rein, A., Levin, J.G., Bassin, R.H., Benjers, B.M., Kashmiri, S.V.S., Hopkins, D. and O'Neill, B.J.: Mutant of B-tropic murine leukemia virus synthesizing an altered polymerase molecule. J. Virol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00070-19 LMG

PERIOD COVERED
October 1, 1978-September 30, 1979

TITLE OF PROJECT (80 characters or less)
Morphogenesis of Animal Viruses During Infection of Mammalian Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jacob V. Maizel, Jr.	Research Chemist	LMG NICHD
OTHERS:	Margaret Stewart	Visiting Associate	LMG NICHD
	Kenneth McClain	Research Associate	LMG NICHD
	Thomas Storch	Research Associate	LMG NICHD
	Ronald Lundquist	Staff Fellow	LMG NICHD
	Robert Lenk	Staff Fellow	LMG NICHD

COOPERATING UNITS (if any)

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LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Molecular Structure

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.2	5.2	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Using mammalian virus systems and combined electron microscopic and biochemical methods, in vivo intermediates in poliovirus translation have been detected and isolated, in vitro poliovirus RNA replication intermediates have been isolated and characterized, new early proteins of adenovirus have been localized, and high resolution molecular mapping of viral genomes has been performed.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Our objective is to describe in molecular details the components of animal viruses and infected human cells with the aim of thereby pinpointing the molecules that cause the genetic and non-genetic changes in transformation and infection, and providing clues to their roles. The animal virus systems used as models have been poliovirus, adenovirus and murine oncornaviruses.

Methods Employed: Each virus system has been examined with the techniques of isotopic tracer analysis in vivo and SDS gel electrophoresis to detect all of the viral proteins and nucleic acids as they are newly synthesized and modified and to detect changes in cellular proteins. In combination with subcellular fractionation techniques individual proteins can be followed through their synthesis and assembly into multimeric forms, and localization in subcellular fractions. By such fractionation powerful clues as to the structural and functional roles are obtained. Electron microscopy is used to characterize individual and complex macromolecules and to localize biologically active molecules through the use of radioautography and immuno-electron microscopy.

Major Findings:

1. Poliovirus - A map of the oligonucleotides from the poliovirus genome has been refined to include a total of 52 different oligonucleotides. The procedure of choice continues to be partial digestion of poliovirus RNA by RNaseIII followed by selection of various length segments starting from the 3' end. Refinement was achieved largely through use of more sensitive analytical procedures, permitting the use of RNaseIII fragments in important length ranges but low yield. Increased sensitivity was obtained by a two-dimensional vacuum elution procedure in which the oligonucleotide fingerprints are in most cases quantitatively transferred to DEAE paper. Use of the DEAE paper in conjunction with a phosphor film increases the sensitivity by 50 to 100-fold. Furthermore, the oligonucleotides can be precisely and conveniently cut out of the DEAE and quantititatively eluted. Identities of oligonucleotides and analysis of mixed fractions was achieved by further digestion with pancreatic RNAse and thin-layer electrophoresis on polyethylene imide sheets.

RNA oligonucleotide maps of defective interfering particles show that at least 11 oligonucleotides are involved in the deletions and that the position in the oligonucleotide map falls within the range for deletions obtained by EM heteroduplex mapping.

Ribosome binding sites in the poliovirus genome have been located using reticulocyte ribosomes and soluble factors either in crude condition or as reconstructed systems. The sites have been characterized as to number,

position and composition by EM mapping and by isolation of RNA fragments protected against nuclease digestion by association with ribosomes. At least four sites have been resolved by EM analysis and at least two have been detected by nuclease protection. A site close to the 5' end and one approximately 600 nucleotides from the 3' end have been found both by EM and by oligonucleotide procedures. Internal sites are clearly present by EM mapping. Protected fragments other than the two major ones but having the ribosome binding properties are present but are less well characterized. Other bound oligonucleotides having binding properties different from translation initiation sites have also been found. At least three of them correspond to oligonucleotides mapped at well defined sites distributed along the genome.

Translation of RNA fragments shorter than the genome and selected to have the 3' ends have shown that functional translation can occur with RNA lacking the 5' end and that it appears to represent polypeptides recognizable as similar to in vivo polypeptides. This may modify the dogma that poliovirus RNA is (only) translatable as a polyprotein.

2. Adenovirus - The position of the gene for the 19K early glycoprotein has been shown not to be in early transcribed region 4 as previously believed from work of others and is likely to be in region 3. Peptide maps have shown that the 15K protein made under conditions of inhibited glycosylation has a very similar polypeptide moiety to the 19K glycoprotein and is distinct from other early and late proteins. Peptide mapping was done on the protein made in vitro that was believed to be 19K and have shown that it is not related. Studies on proteins made from mutant adenovirus containing deletions and SV40 insertions in the early region 3 have confirmed the above observations.

Preparations of the mild detergent-lysed adenovirus-infected cells have been prepared and analyzed by EM and gel electrophoresis. Morphologically the cytoskeletons of these cells show significant changes. They retain some of each of the early adenovirus-induced proteins, including the 19K glycoprotein. Since 19K has also been found enriched in non-detergent-treated plasma membranes it is suggested that 19K resides in the membrane with attachments to the cytoskeleton.

3. Methodology - Computer analyses of nucleic acid sequence data have progressed with the development of a graphic matrix method for revealing patterns of similarity or self-complementarity. Numerical matrix methods have existed but are limited by the huge composition of computer time and memroy. The graphic method stores only small portions of a matrix and displays the data as a dot matrix having a linear density of about 80 dots per inch. Thus a comparison of 1000 sequences of 1000 bases needs 1,000,000 storage elements or in this graphic method is a square 33 cm on a side. In this way every base can be compared to every other base in sequences of considerable size. The result of regions of similarity is a diagonal line which is almost immediately apparent. At a glance it is apparent where

parts of genes have been duplicated, where insertions or deletions have occurred, where high evolutionary separation has occurred and how all such events are related to others at different places in the molecule. The method has been successfully applied to globin, immunoglobulin and other systems. By simple alteration in the procedure, it is possible to recognize regions of inverted repeat and to see their relationship to each other. It is possible to detect good candidates for the formation of regions of secondary structure in nucleic acids and one such possibility has been demonstrated by EM and graphic matrix methods in the mouse immunoglobulin constant region. Programs to conveniently produce graphic matrices have been designed to allow selection display of certain types of regions and to display the actual base sequences involved through the use of four-color graphic matrices. Methods to measure and classify nucleic acids in electron micrographs continue to be developed with Dr. Lipkin's group. A program to trace simple hairpin and tree-like structures is functional. Work to allow measurement of loop-containing structures has begun.

As mentioned above methods for biochemical mapping of nucleic acids have been greatly refined through the use of more rapid and sensitive techniques. Mainly the quantitative vacuum transfer of two-dimensional acrylamide gels onto DEAE paper from which fragments can be detected and those eluted in pure form, free of gel impurities and suitable for enzymatic treatment. A fingerprint that once required approximately 2-600,000 cpm of ^{32}P for wet radiograph to appear overnight can now be obtained with 40-80,000 cpm. A complete recovery of 50 oligonucleotides and subsequent pancreactic nuclease digest and PEI-electrophoresis can be done with 250,000 cpm in three days.

Proposed Course of Project: We will continue EM, biochemical and computer techniques to characterize molecules and relate them to the structure and workings of cells. Efforts will be continued to devise similar EM tags for molecules and to use them in mapping molecules and cells. Work will continue to devise ways to reveal information contained in nucleic acid sequences.

Significance to Biomedical Research and the Program of the Institute: Biochemical characterization of the early events in viral infections has more completely defined the critical events in cellular and viral regulatory mechanisms. New techniques using electron microscopy are now permitting visualization of molecules in the process of carrying out their regulatory roles. Observations of the specific interactions between cellular and viral genomes and their transcriptional and translational products strikingly verify and extend biochemical findings. Information thus derived will find application in control of reproduction, growth, differentiation and viral disease in higher organisms.

Publications:

1. Lundquist, R.E. and Maizel, Jr., J.V.: *In vivo* regulation of the poliovirus RNA polymerase. *Virology* 89:484-493, 1978.

2. Lundquist, R.E., Sullivan, M. and Maizel, Jr., J.V.: Characterization of a new isolate of poliovirus defective interfering particles. *Cell*, in press.

3. Shapiro, B., Lipkin, L. and Maizel, J.V., Jr.: Computerized generation of secondary structure maps for nucleic acids. *Computers in Biomedical Research*, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00071-07 LMG

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)
Study of Adenovirus Gene Functions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. Westphal	Research Geneticist	LMG NICHD
OTHERS:	G. Kitchingman	Research Associate	LMG NICHD
	W. Richardson	Visiting Fellow	LMG NICHD

COOPERATING UNITS (if any)
a) Tumor Virology Laboratory, Salk Institute, San Diego, Calif.
b) Laboratory of Molecular Biology, NINCDS, NIH
c) Department of Cell Biology, Roche Institute of Molecular Biology, Nutley, N.J.

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Animal Viruses

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study is the analysis of the instruments and controls of adenovirus gene expression in lytically infected or in transformed cell cultures. Topics currently under investigation include: (A) processing of early adenovirus RNA. (B) analysis of early gene function, (C) virus gene structure and expression as a function of host cell genetic environment. Besides standard procedures of molecular genetics and biochemistry, techniques such as micro-injection of animal cells, electron microscopy and gene cloning in prokaryotic vectors, are used in these studies.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Our group studies the instruments and controls of viral gene expression in cultured animal cells.

Methods Employed: See Summary.

Major Findings: The phenomenon of RNA splicing has continued to be the focal point of our studies. Following our detection of intervening sequences in early adenovirus genes, we have used the electron microscope to determine the structures of nuclear and cytoplasmic RNAs derived from the four early regions of adenovirus type 2 DNA. Our data are consistent with the idea that cytoplasmic RNAs are derived from nuclear precursors by a "cut-and-splice" mechanism. Moreoever, they allow us insights into the intricate controls of early virus gene expression.

In collaboration with colleagues of the Salk Institute in San Diego, we examined the structure and expression of SV40 sequences present in the non-defective hybrid virus $Ad2^{+}ND4$. An interesting and most unexpected outcome of these studies is the fact that monkey and human cells differ in functions (possibly splicing enzymes) that mediate the arrangement and expression of these SV40 sequences.

Electron microscopy of vesicular stomatitis virus RNA has been the topic of a joint study with colleagues of NINCDS and the Roche Institute at Nutley. The messages of this RNA virus have been mapped. Interestingly, the mRNAs are occoasionally linked at intercistronic boundaries by stretches of polyadenylic acid. This phenomenon is under continued study at NINCDS.

Significance to Biomedical Research and the Program of the Institute: Our studies are designed to contribute to an understanding of the molecular events which accompany cell infection by a virus that is known as a human pathogen in childhood diseases, as well as a tumor virus in rodents. As shown above, our experiments offer insights into basic mechanisms of genetic control and regulation in eukaryotic cells.

Proposed Course of the Project: (a) Early adenovirus genes will be amplified in prokaryotic vectors. Part of the material will be used for DNA sequencing. Also, efforts will be made to introduce individual early genes into suitable mammalian host cells by transfection or microinjection. We hope to obtain a set of permanent cell lines that actively express one gene at a time. This would greatly aid studies of early gene function and regulation. (b) Studies of host functions that mediate the arrangement and expression of $Ad2^{+}ND4$ genes will continue in collaboration with colleagues at the NINCDS. (c) A joint project with colleagues at the NIAMDD concerns the nature of adenovirus gene functions required to promote the growth of the defective adeno-associated virus (AAV), as well as the structure of AAV RNAs.

Publications:

1. Herman, R.C., Adler, S., Lazzarini, R.A., Colonno, R.J., Bannerjee, A.K. and Westphal, H.: Intervening polyadenylate sequences in RNA transcripts of vesicular stomatitis virus. Cell 15: 587-596, 1978.

2. Westphal, H., Lai, S.-P., Lawrence, C., Hunter, T. and Walter, G.: Mosaic adenovirus-SV40 RNA specified by the nondefective hybrid virus $Ad2^{+}ND_4$. J. Mol. Biol. 130: 337-351, 1979.

3. Westphal, H., Kitchingman, G.R., Lai, S.-P., Lawrence, C., Hunter, T. and Walter, G.: Splice patterns of adenovirus and adenovirus-SV40 mosaic RNAs. The Eleventh Miami Winter Symposium, Miami, Florida, University of Miami and the Papanicolaou Cancer Research Institute, 1979, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00072-12 LMG

PERIOD COVERED

October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Crystallin Synthesis in Development and Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Joram Piatigorsky	Biologist	LMG NICHD
OTHERS:	Toshimichi Shinohara	Staff Fellow	LMG NICHD
	Raymond E. Jones	Staff Vellow	LMG NICHD
	Suraj P. Bhat	Visiting Fellow	LMG NICHD
	Harry Ostrer	Clinical Associate	LMG NICHD

COOPERATING UNITS (if any)

Peggy Zelenka	Laboratory of Vision Research, NEI		
Peter F. Kador	"	"	"
Jin H. Kinoshita	"	"	"

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Section on Cellular Differentiation

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	5.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our goal of understanding crystallin gene expression during development and disease has been advanced as follows. More evidence has been obtained that electrolyte imbalances may alter crystallin metabolism in cataracts. Differential reduction in β- and γ-crystallin synthesis and crystallin degradation have been found in a new hereditary mouse cataract (Philly mouse) associated with ion changes. Na^+, K^+, Cl^- and OAc^- were shown to effect the ratio of synthesis of the δ-crystallin polypeptides in a reticulocyte lysate as well as in the cultured chick lens treated with ouabain, providing direct evidence that electrolytes may alter crystallin systhesis. Experiments have indicated that the ion-related changes in δ-crystallin synthesis may involve a partial cleavage of the larger polypeptides associated with translation or a direct effect of ions on initiation or termination of translation. We have correlated subunit composition with isoelectric points of δ-crystallin, providing a basis for the charge heterogeneity of the protein. δ-Crystallin gene sequences have been cloned; 16 intervening sequences have been detected in the δ-crystallin genomic fragments. Finally, chicken β-crystallins have been purified and their polypeptide composition studied with respect to size and tryptic peptides.

Project Description:

Objectives: To describe the normal sequence of events at the molecular level of δ-crystallin gene expression during differentiation of embryonic chick lens cells, and to understand the regulation of protein metabolism in clear and cataractous lenses.

Methods Employed: Conventional techniques of nucleic acid and protein biochemistry are applied.

Major Findings:

1) Previously, we have correlated changes in the intracellular concentrations of Na^+ and K^+ with differential alterations in crystallin metabolism during cataractogenesis. This year we have extended this correlation to a new hereditary cataract in the mouse (Philly cataract). The Philly cataract is particularly interesting since, in contrast to other cataracts, α-crystallin synthesis persists as β- and γ-crystallin synthesis are differentially reduced.

2) Earlier we showed that the ratio of synthesis of the δ-crystallin polypeptides, which have molecular weights near 50K and 48K, is influenced by the intracellular concentrations of Na^+ and K^+. This year we have demonstrated that this alteration in δ-crystallin synthesis can be reproduced in a reticulocyte lysate primed with δ-crystallin mRNA. In addition, anions (Cl^- and OAc^-) were shown to affect the ratio of synthesis of the 50K to the 48K δ-crystallin polypeptides in the cell-free system and in the cultured embryonic chick lens treated with ouabain. These experiments provide direct evidence that electrolytes may have a differential effect on δ-crystallin synthesis. We have demonstrated that the 50K δ-crystallin polypeptides have two methionine-containing tryptic peptides not present in the 48K polypeptides. Limited trypsin-treatment of native δ-crystallin removed these two tryptic peptides and converted the 50K into the 48K species. Taken together, our data are consistent with an ion-dependent, partial cleavage of the 50K polypeptides coupled with translation or with a direct effect of these ions on translation.

3) We have shown that δ-crystallins with different isoelectric points have different subunit compositions. This provides the major reason for the multiple isoelectric points of δ-crystallin.

4) Strong evidence has been obtained indicating that cytoplasmic δ-crystallin contaminates preparations of membranes from the lens. This questions seriously the current idea that crystallins are intrinsic membrane components.

5) δ-Crystallin cDNA has been cloned in a bacterial plasmid and the δ-crystallin sequences partially characterized. The cloned cDNA provides a major analytical probe for our studies on δ-crystallin gene expression during development.

6) Restriction enzyme analysis of δ-crystallin sequences in the chicken genome has provided evidence for the existence of two δ-crystallin genes or two alleles of this gene. We have cloned most of the δ-crystallin genomic sequences in λ Charon 4A and have demonstrated the existence of at least 16 intervening sequences in the cloned fragments.

7) We have initiated a study concerning β-crystallin gene expression during lens development and maturation. As a first step, six chicken β-crystallin polypeptides have been identified and shown to possess similar tryptic fingerprints, suggesting similarity in primary structure. Aggregates with different combinations of β-crystallin polypeptides were found in lens extract.

Significance to Biomedical Research and the Program of the Institute: These studies contribute to our knowledge of the mechanisms of cellular differentiation and disease, especially with regard to the mechanisms of specific protein synthesis in developing and cataractous lenses. In addition the detailed analysis of the lens crystallins will ultimately further our understanding of lens clarity and the loss of clarity, as occurs in cataracts.

Proposed Course of Project: Crystallin mRNA levels will be quantitated in several mammalian cataracts known to be associated with differential reductions in crystallin synthesis. These studies will involve molecular cloning of mammalian crystallin DNA sequences. Further analysis will be performed on the δ-crystallin gene sequences in chickens. The organization and structure of δ-crystallin gene sequences will be compared in duck and chickens. Attempts will be made to isolate and clone β-crystallin mRNA gene sequences.

Publications:

1. Piatigorsky, J.: Differences in isoelectric points and subunit compositions of δ-crystallins synthesized in lenses cultured with and without vitreous bodies. Exp. Eye Res. 27: 227-237, 1978.

2. Williams, L.A., and Piatigorsky, J.: Heterogeneity of δ-crystallins of the embryonic mallard lens. Correlation between subunit composition and isoelectric points. Biochemistry 18: 1438-1442, 1978.

3. Piatigorsky, J.: Intracellular ions, protein metabolism and cataract formation. In Zadunaisky, J. and Davson, H. (Ed.): Current Topics in Eye Research, Vol. 2, in press.

4. Piatigorsky, J., Shinohara, T., Bhat, S.P., Reszelbach, R., Jones, R.E., and Sullivan, M.A.: Correlated changes in δ-crystallin synthesis and ion concentrations in the embryonic chick lens: Summary, current experiments and speculations. Ann. N.Y. Acad. Sci., in press.

5. Zelenka, P., Reszelbach, R. and Piatigorsky, J.: Developmental changes in proteins of purified membranes of chicken lenses and evidence for contamination by cytoplasmic δ-crystallin. Biochim. Biophys. Acta, in press.

6. Shinohara, T., and Piatigorsky, J.: Age-dependent differences in the ratio of synthesis of δ-crystallin polypeptides in cultured vitreous-free lenses of chick embryos. Develop. Biol., in press.

7. Bhat, S.P., and Piatigorsky, J.: Molecular cloning and partial characterization of δ-crystallin cDNA sequences in a bacterial plasmid. Proc. Natl. Acad. Sci., USA, in press.

8. Williams, L., and Piatigorsky, J.: Comparative and evolutionary aspects of δ-crystallin in the vertebrate lens. Eur. J. Biochem., in press.

9. Piatigorsky, J., Kador, P.F., and Kinoshita, J.H.: Differential synthesis and degradation of protein in the hereditary Philly mouse cataract. Exp. Eye Res., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00073-08 LMG

PERIOD COVERED

October 1, 1978 through October 1, 1979

TITLE OF PROJECT (80 characters or less)

Regulation of Antibody Response at the Cellular Level

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Edgar E. Hanna	Microbiologist	LMG NICHD
OTHER:	Michael L. Misfeldt	Staff Fellow	LMG NICHD

COOPERATING UNITS (if any)

C. T. Hansen, Geneticist, VR, DRS, NIH

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Section on Gene Organization and Control

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.1	2.0	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Experiments are directed toward understanding processes by which regulation of the immune system is controlled at the cellular level. The integrated roles of regulatory cells such as thymus-derived helper and suppressor T-cells, bone-marrow derived antibody-forming B-cells and accessory macrophages are being delineated. Specifically we are seeking an understanding of the relationship of suppressor and helper cells and their modes of regulating antibody-forming B-cells. We have demonstrated that a streptococcal exotoxin (SPE) inhibits preferentially an activity which has been ascribed to suppressor cells. SPE is therefore potentially a useful probe for the study of suppressor cells. Sub-populations of immunocytes fractionated from T-cell sufficient +/nu donor mice are treated appropriately with SPE or other mitogens in vitro. The cells are then tested for their capacity to complement the antibody responses of T-cell deficient nude (nu/nu) mice or their spleen lymphocytes (B-cells) in vitro. This study involving SPE may also derive relevant information about the sequelae of acute streptococcal disease, as well as fundamental information on the cellular biology of the immune system.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To understand the processes by which regulation of the immune system is controlled at the cellular level. Specifically, we seek to understand the mechanisms by which thymus-derived T-cells regulate antibody formation and secretion by interaction with bone marrow derived precursor cells (B-cells) of antibody-forming cells. We seek to understand the relationship between helper T-cells which augment the B-cell response and suppressor T-cells which curtail the B-cell response. We seek also to understand the accessory role of macrophages in the activities of both cell types. It remains unclear whether helper cells and suppressor cells are distinct cell types or the same cells expressing different phenotypes in response to different stimuli--or whether such different phenotypes may be reflecting stages of development and maturation of a single cell type. Accordingly, we seek to understand the biological and molecular mechanisms of interaction between either T-cell and the B-cell. We wish to discern the nature of the intracellular site or structures on which SPE appears to localize after it is absorbed to the membranes of T-cells. The molecular nature of the membrane receptor for SPE which appears now to be present on all cells albeit active only on certain cells of the immune system remains to be elucidated.

Methods Employed: Major advantage is taken of our ability to perform cellular reconstruction (complementation) experiments in vitro. This procedure involves the use of nude mouse B-cells and macrophages to which we can add selectively, variously isolated and treated T-cells from immunocompetent +/nu littermate donor mice. We can subsequently test whether complete or deficient antibody responsiveness is conferred upon B-cells from the nude mice. Similarly, macrophages may be separated from nude cell populations, which are uncontaminated with T-cells, treated as required, added back or withheld from the system in order to test macrophage functions. Immunofluorescence is the method used to discover and follow the location of SPE on and within cells to which it adsorbs. Plasmacytoma and/or T-lymphoma to lymphocyte cell fusion techniques are being adapted for the construction of hybridoma cell lines in order to perpetuate the various subpopulations of immunocytes. General and routine methodology has been detailed inn previous reports.

Major Findings: Our studies have demonstrated that the target cell affected by SPE that deregulates an antibody response is a T-cell. Previous data have indicated that the suppressor class of T-cells is the particular subpopulation uniquely affected by SPE. However, delay of the function of helper T-cells could also give a response similar to one generated as a result of inactivation of suppressor T-cells. Very recent results continue to favor the hypothesis that SPE inactivates or modulates the function of a unique class of T-cell. T-cells have been fractionated on solid-phase (nylon wool) columns into two populations, pool 1 and pool 2. SPE treatment of pool 2 cells leads to the generation of the SPE effect which has been termed a deregulated response. Treatment of pool 1 cells results in an early and transient suppression of the immune system. Pool 1 cells were

observed to be Lyl^+ cells whereas pool 2 cells were observed to be largely $Ly2^+$ as determined by immunofluorescence. Similar fractionations of T-cells from the spleens of mice primed with immunogen-carrier (e.g. RE for TNP-RE) results in cells of pool 1 becoming refractory to suppression by SPE. Pool 2 cells from similarly primed spleens continue to generate the SPE effect. Pool 1 consists of T-cells which exert a positive (helper) regulatory function on B-cells. This function was shown to increase proportionally to an optimum during the addition of graded numbers of Pool 1 T-cells to a constant optimal number of B-cells. Pool 2 appears to consist of several subpopulations of more adherent T-cells which cannot be separated from each other on the nylon wool columns. However, they are obviated by their polyphasic, but unseparable elution peaks from the nylon wool columns. We observe that at least one of the Pool 2 subpopulations is capable of shutting off the antibody-forming function of the B-cell population. This suppressor function increases proportionally as graded numbers of Pool 2 cells are added to an optimally complemented, constant number of B-cells. We observe that this suppressor activity of a Pool 2 subpopulation is inhibited by SPE. SPE pretreated donor splenocytes were also fractionated into Pool 1 and Pool 2 subpopulations. By this procedure, both Pool and Pool 2 T-cells supported a delayed burst of PFC (SPE generated deregulation). An altered elution profile of Pool 1 and Pool 2 helps to suggest that SPE also drives this new activity into Pool 1 by affecting a shift in or development of the activity of another of the Pool 2 subpopulations.

Experiments designed to discover an SPE-specfiic receptor on a unique class of T-cells indicates that SPE binds to all cells of the immune system, albeit, with different distribution properties. Macrophages imbibe SPE; B-lymphocytes bind SPE as large patches at their membranes. These are polarized, "capped" rapidly (depending on the molecular nature of SPE) and appear to be shed by B-lymphocytes. Less purified SPE, which appears to be a more native molecule appears to be much more avid toward inducing this capping phenomenon on B-lymphocytes. T-lymphocytes bind SPE in a diffuse pattern and do not readily cap SPE. Instead SPE appears to progress from a diffuse localization to intracellular sites within T-cells.

Significance to Biomedical Research and the Program of the Institute:
Understanding the natural mechanism of the regulated expressions of the immune system may reveal pathways by which defective immune systems may be corrected. The potential ability to deregulate and to re-organize individual immune systems is highly relevant to cures for certain diseases. SPE is known to be a metabolite of only the pathogenic Group A streptococci. Our demonstration that SPE can deregulate immune systems may be relevant to an understanding of the mechanism of certain autoimmune sequelae of acute streptococcal infections. Furthermore, results of our study should complement scientific thought on cellular control mechanisms in general.

Proposed Course of Project: We are constructing hybridomas between mouse plasmacytomas, mouse T-cell lymphomas and fractionated mouse splenocytes (Pool 1, Pool 2 T-cells; B-cells and macrophages). Special attention is being devoted toward perpetuating the discrete subpopulations of Pool 2 with the expectation that we will derive hybrid T-cell lines with activities which are blocked and maintained at various levels of development from the precursor stage to the helper stage. As this work progresses our discovery more precisely of the exact site of action of SPE and regulation of the immune system will be facilitated.

In our related experiments on the two phenotypes of nude mice as defined by their differences in T-cell deficiency and levels of T-cell precursors, we are attempting to fractionate the T-cell precursors. We will attempt to perpetuate these precursor cells as cell lines by the hybridoma technique as described above. Hopefully we will in the course of these related experiments in concert, derive cell lines capable of performing all of the functions required for a complete immune system.

Publications:

1. Hanna, E.E. and Hale, M.L.: Complementation of the anti-TNP PFC response of N:NIH nude mice from nude dams by spleen cells of nude mice from heterozygous-for-nude dams. Cellular Immunology, in press.

2. Hanna, E.E.: Hybridomas: Fusion of lymphocytes and mouse plasmacytomas--an approach to specific monoclonal antibodies. Introduction and Scope. In D. Schlessinger (ed.), Microbiology-1980, American Society for Microbiology, Washington, D.C., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00074-08 LMG

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)
Regulated Gene Expression During Cell Growth and Differentiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. Leder	Chief	LMG NICHD
OTHERS:	J.G. Seidman	Staff Fellow	LMG NICHD
	E.E. Max	Research Associate	LMG NICHD
	M. Kaehler	Staff Fellow	LMG NICHD
	D. Konkel	Staff Fellow	LMG NICHD
	H. Miller	Research Associate	LMG NICHD
	Y. Nishioka	Visiting Fellow	LMG NICHD

COOPERATING UNITS (if any)
D. Hamer, NIAID

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Gene Organization and Control

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
8.5	6.5	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are attempting to define the molecular basis of gene expression in higher organisms. Two model genetic systems, globin and immunoglobulin, are being used for this purpose. The work has involved recombinant DNA technology and has resulted in the cloning of several mouse globin and immunoglobulin genes whose nucleotide sequences now have been completely determined. These studies have permitted us to identify certain regions of the globin gene that are concerned with gene transcription and RNA splicing. We have also cloned the mouse globin genes in the simian virus SV40 and forced their accurate expression in infected cells, thus proving the universality of transcriptional and splicing mechanisms. In addition, the structural studies of the immunoglobulin genes indicate that immunoglobulin light chain gene diversity arises from hundreds of germline variable region genes that undergo recombination with one of five J- or joining segments adjacent to one germline constant region gene.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Our objective is to understand, in molecular terms, the mechanisms which regulate the flow and exchange of genetic information during cell growth and development. We use two model systems, one involving the process of erythrodifferentiation, the other, immunodifferentiation. Specifically we ask how genetic elements corresponding to globin and immunoglobulin are organized in the genome, how they arise during evolution, what elements in the genome and what gene products regulate their expression and how this regulation and organization are relevant to other elements in the genome of higher organisms.

Methods Employed: These investigations have, naturally, involved in interdisciplinary approach. In addition to the usual techniques of cell growth and culture and the biochemistry of nucleic acids and proteins, special attention has been given to the development of a special biological containment facility called a P3 facility as well as specific techniques newly developed for the manipulation of recombinant DNA molecules and their detection.

Major Findings:

1. These investigations have now lead to the cloning of all the adult alpha and beta globin genes of the mouse.

2. The complete nucleotide sequences of mouse beta major, beta minor and an alpha gene have been determined. The preservation of gene interruptions indicates that the ancestral gene was interrupted and that these intervening sequences have been preserved since early vertebrate evolution.

3. Sequence correlation analysis has allowed us to define two models of evolution of these genes, a fast and a slow one, involving insertion-deletion and point alterations, respectively.

4. The mouse globin genes have been cloned in SV40 and expressed in infected African green monkey kidney cells, proving the universality of transcriptional and RNA splice mechanisms.

5. Analysis of the cloned and sequenced immunoglobulin variable, J and constant region genes has established the major source and mechanism for generating immunoglobulin diversity. This involves hundreds of germline genes that undergo somatic translocation to one of a series of five joining or J segments close to a single constant region gene sequence.

Significance to Biomedical Research and the Program of the Institute:
These studies are directed towards understanding fundamental processes involved in the expression of genetic information. They are, thus, obviously relevant to achieving an understanding of a variety of inherited disorders

that afflict man. By inherited disorders we refer not only to clearly identifiable conditions which involve mutations at a single genetic site, but also polygenic disorders such as diabetes, hypertension and susceptibility to oncogenic disease. For example, our studies in the past have led to techniques which have permitted a far more detailed understanding of the inherited anemia, thalassemia. Current studies involving gene cloning are obviously relevant to the possible production of valuable pharmacologic reagents as well as the diagnosis of certain inherited diseases such as thalassemia and sickle cell anemia.

Proposed Course of Research: Efforts over the next year will be directed further towards understanding of the organization of globin and immunoglobulin genes. We will complete the sequences of the adult globin genes and complete cloning and sequencing of the embryonic genes. We will exploit the globin/SV40 hybrids to introduce specific mutations that affect gene transcription and processing in order to establish the molecular basis of each. We shall extend our studies of the immunoglobulin genes to determine the precise basis of somatic recombination. These studies will also be extended to the mouse heavy chain and the problem of the heavy chain switch. In addition, we shall clone and determine the organization of human immunoglobulin genes for the dual purpose of evolutionary comparison and in order to understand certain inherited disorders of immunoglobulin biosynthesis.

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